

Understanding Genotoxic Impurities and Degradants

**Impact on Science and Compliance
Strategies**



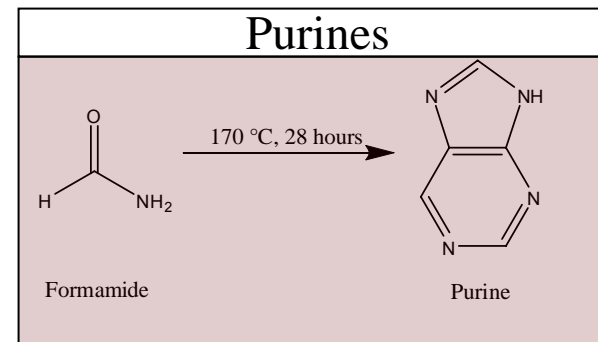
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Presentation Outline

- Understanding Genotoxicity
 - Sources of Genotoxicity
 - Genotoxic Impurities
- Understanding the Regulatory and PhARMA Positions
 - EMEA, PhARMA and FDA
- Understanding Identification and Testing Options for Genotoxic Impurities and Degradants
 - *In Silico*, *In Vitro* and *In Vivo* Approaches
- Cautionary Tale



Guiding Thoughts

- “Although medicinal products are required to be safe, safety does not mean zero risk. A safe product is one that has reasonable risks, given the magnitude of the benefits expected and the alternatives available.”
(unknown)
 - Do the regulations make sense?
- Of 467 drugs listed in the 1999 PDR, a survey of open literature found approximately 75% have at least one genotox (mostly *in vitro*) result.
 - Snyder, R.D., and J.W. Green. 2001. A review of the genotoxicity of marketed pharmaceuticals. *Mutat. Res.* 488:151–169.
 - The drug development process does not stop with a single genotoxic result!

Pyrimidines

Genotoxicity

- **Genotoxic**

- Main Entry: **ge·no·tox·ic**

Pronunciation: j -n - tāk-sik

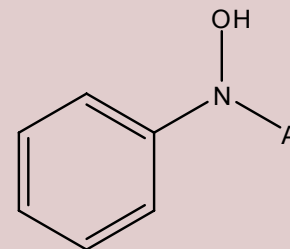
Function: *adjective*

: damaging to genetic material <environmental exposure to *genotoxic* agents -- P. A. Gaspar *et al*>

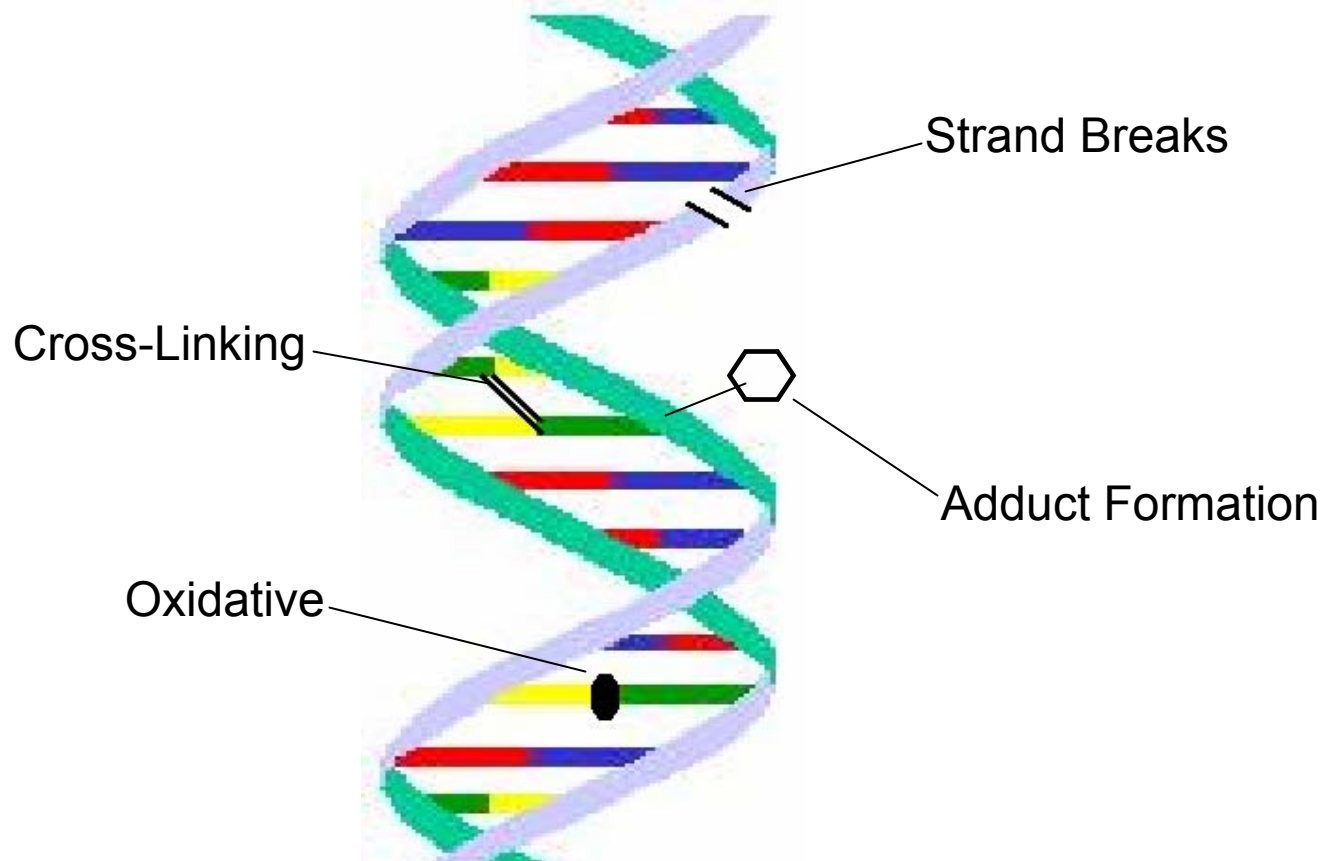
- **ge·no·tox·ic·i·ty** /-tāk- sis- t- / *noun, plural –ties*

Merriam-Webster Medical Dictionary

N-Hydroxyaryls



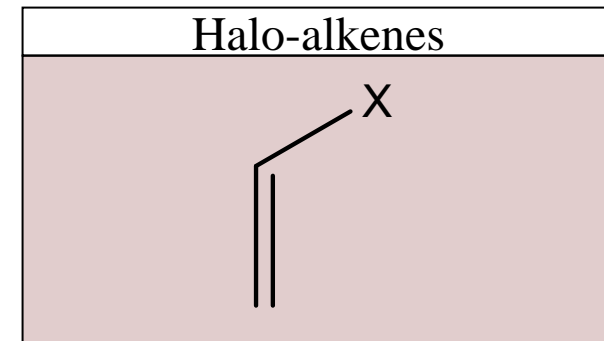
Types of DNA Damage



The human genome consists of approximately 3×10^9 bases, in a sequence produced from only four bases, namely adenine (A), cytosine (C), guanine (G) and thymine (T).

Types of Genetic Damage

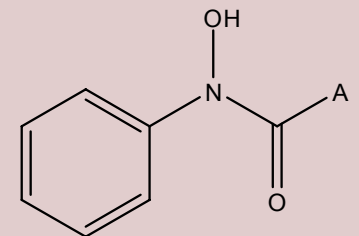
- Mutagenic (not all DNA damage results in mutation)
 - Causing change in the genetic information
 - Senescence – irreversible dormancy
 - Apoptosis – programmed cell death
 - unregulated cell division
- Clastogenic
 - Causing change in the chromosome
 - Structural (breaks)
 - Numerical (aneuploidy) – changes in the number in a cell
- Carcinogenic
 - Causing or facilitating propagation of cancer
 - Sufficient mutagenic and/or clastogenic activity = carcinogenicity



Exogenous sources of DNA damage

- Radiation
 - UV (200 – 300 nm) radiation
 - X-ray, gamma ray
- Hydrolysis or thermal disruption
- “Natural” toxins – plant/animal sources
- Mutagenic chemicals – human sources
- Medicinal
 - Chemotherapy
 - Radiation Therapy

N-Acylated aminoaryls



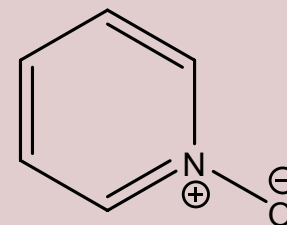
DNA Targeting Anti-Cancer Drugs

Cyclophosphamide	Cytoxan®	alkylating agents; form interstrand and/or intrastrand crosslinks
Melphalan	Alkeran®	
Busulfan	Myleran®	
Chlorambucil	Leukeran®	
Mitomycin	Mutamycin®	
Cisplatin	Platinol®	forms crosslinks
Bleomycin	Blenoxane®	cuts DNA strands between GT or GC
Irinotecan	Camptosar®	inhibit the proper functioning of enzymes (topoisomerases) needed to unwind DNA for replication and transcription
Mitoxantrone	Novantrone®	
Dactinomycin	Cosmegen®	inserts into the double helix preventing its unwinding

Impurities

- No therapeutic benefit, only risk
- Represent contamination and lack of process control
- Inevitable
 - Often avoidable (reagent change, route change)
 - Often controllable (environmental controls, purge strategies, preservatives, etc.)
- Degradants
 - Specific type of impurity
 - Everything above applies...

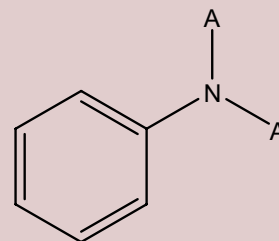
Aza-aryl N-Oxides



Current Structural Alerts of Interest

- DNA adduct and cross linking formation
 - Preventing accurate replication
 - The DNA bases are electron rich and can act as nucleophiles
 - Deoxyguanosine, deoxyadenosine, deoxycytidine, and deoxythymidine
 - Potentially interact with
 - Epoxides
 - Aryl- and aromatic-amines
 - Aryl nitro compounds
 - Primary halides
 - **Alkylsulfonate esters**
 - Alkyl esters
 - Aldehydes (particularly aromatic)

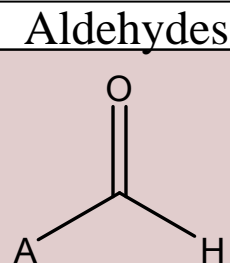
Aminoaryls



Regulation and Control of Genotoxic Impurities

In the Beginning...

- Delaney Clause (1958)
 - Amendment to Food, Drugs and Cosmetic Act (1938)
 - No food or color additive could be approved if found to cause cancer in man or animals
- Threshold of Toxicological Concern (TTC)
 - Established by FDA as Threshold of Regulation for food packaging leachables
 - Negligible risk =
 $0.5 \text{ ppb} \times 3000 \text{ g/day} = 1.5 \text{ } \mu\text{g/day}$

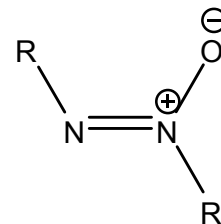
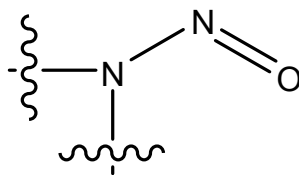
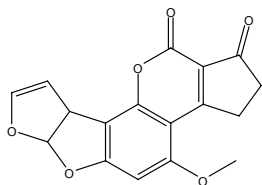


European Medicines Agency (EMA)

- 2002 – Initial Draft
- 2007 – Effective
- Supplements ICH Q3A, B and C guidelines
- Applies only to Marketed Products
- For substances with threshold mechanisms, must control to that level
- For substances without threshold mechanisms, apply the TTC concept and the 1.5 µg/day intake limit
- Exceptions to TTC
 - Highly genotoxic compounds (aflatoxin, nitroso amine, azoxy)
 - End of life, short term exposure, particularly important disease states
 - Not clearly defined for early phase development
- The Big Question: is this TTC reasonable?
 - Original definition of TTC based on Negligible Risk now taken as Acceptable Risk for a beneficial drug
 - TTC assumed cancer risk < 1 in 100,000 or < 0.001%
 - Based on 2002 – 2004 cancer rates, 41% of people born today WILL be diagnosed with cancer at some time during their lifetime (National Cancer Institute)

EMEA (continued)

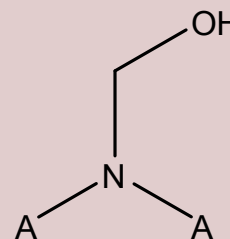
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PhARMA

- Genotoxicity Taskforce –
 - Hoffmann-La Roche, Pfizer, ALZA, GSK, Merck, J&J, Abbott, Noramco, Sanofi-Aventis, AZ, Wyeth, Lilly, BMS
 - Regulatory Toxicology and Pharmacology 44 (2006) 198–211
- Classification System
 - Class 1 – Impurities known to be genotoxic AND carcinogenic
 - Class 2 – Known genotoxic, but unknown carcinogenic potential
 - Class 3 – Structural alert unrelated to API, unknown genotoxicity
 - Class 4 – Structural alert related to API
 - Class 5 – No alerting structure or sufficient evidence for absence of genotoxicity

N-Methylols



PhARMA (continued)

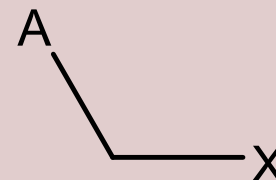
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- Staged TTC based on duration of exposure
 - EMEA limits based on 70 year exposure assumptions

	≤ 1 month	>1–3 months	>3–6 months	>6–12 months	>12 months
Allowable Daily Intake (µg/day) for all phases of development	120	60	20	10	1.5
Alternative maximum level of allowable impurity based on percentage of impurity in API	0.5%	0.5%	0.5%	0.5%	

Issues/Limitations with PhARMA

- What about “professional” clinical subjects?
 - How to control lifetime exposure?
 - Impact on Clinical Enrollment?
- What about multiple genotoxic impurities?
 - If structurally similar should they be counted as one?
 - Should there be an upper limit on the number of genotoxic impurities in a substance or product?

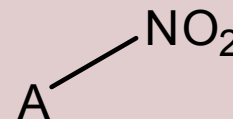
Primary alkyl-, aryl-halides



FDA - Current Thinking

- We're still reading the tea leaves
 - No Clear Published Guidance
 - Likely to concur with EMEA guidance
 - Some evidence that the PhARMA position is being heard!
 - Will it offer clarity on investigational phases of development?

Nitro- compounds



FDA - Current Thinking

□ David Jacobson-Kram

- Office of New Drugs, CDER, FDA
- 2005 Lecture, “Use of Genotoxicity Data to Support Clinical Trials or Positive Genetox Findings on a Candidate Pharmaceutical or Impurity Now What?”
 - Int. J. Toxicol., 24:3, 129-134

“The Food and Drug Administration (FDA) is currently working on a guideline for genotoxic impurities and **considers the reasoning outlined in the EMEA publication to be an excellent starting point** for FDA’s own deliberations. Issues that are currently under discussion include the observations that (1) **carcinogens can vary in potency** by several orders of magnitude; (2) because drugs may contain many impurities, whether there should be an **upper limit to the number of TTCs permitted**; and (3) TTCs are based on lifetime exposures whereas **early clinical trials may be only weeks or months in duration**. **In this latter situation, higher levels of impurities may be acceptable but reaching a consensus on what those levels should be will no doubt be a challenge.**”

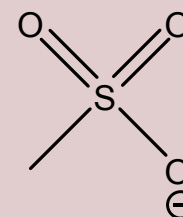
FDA - Current Thinking

- “Regulation of genotoxic and carcinogenic impurities in drug substances and products”
 - Timothy McGovern, David Jacobson-Kram,
 - *Trends in Analytical Chemistry*, Vol. 25 No. 8, 2006

- **“Some flexibility in the threshold level can be applied during the investigational stages, since clinical trials may vary widely in terms of duration from short-term (single dose to 4 weeks) to years and the qualification threshold for a marketing application is based on risk estimates from lifetime rodent assays that incorporate conservative assumptions. Additionally, it is recognized that the pharmaceutical industry may be limited during early developmental stages in its ability to identify and to control drug-related impurities. Higher daily levels may therefore be acceptable during the clinical development stage without significantly increasing the potential risk to trial subjects.”**

- Discussion of controlling multiple, structurally related impurities as if they are a single impurity!

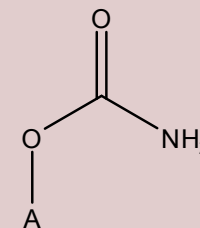
Mesylates



Remaining Regulatory Issues

- Excipients
 - Not specifically governed
 - Who is responsible for testing and control
- Lack of good assays to prove real thresholds in conjunction with DNA repair mechanisms
- Defining ALARA / ALARP
- ALARM!!
 - Analysts Losing All Rational Mindsets
 - As Low As Reasonably Measured
 - Improving Analytical Techniques
 - We have only ourselves to blame!
 - LC/MS/MS
 - Extraction and concentration

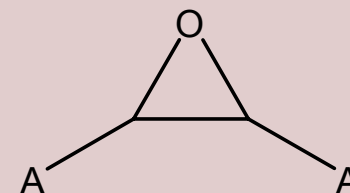
Carbamates (urethanes)



The Tools of Genotoxic Assessment

- How to identify potential genotoxic impurities
- How to assign impurities as potentially genotoxic

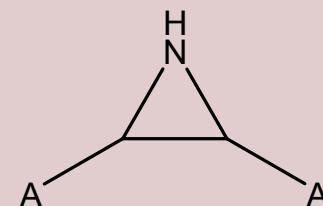
Epoxides



How to Identify Potential Genotoxicity

- Through Historical and Deductive Structural Alerts
 - DEREK (Deductive Estimation of Risk from Existing Knowledge)
 - MCase (Multi Computer Automated Structure Evaluation)
 - TOPKAT (Still looking for what this means!!)
 - CPDB (Carcinogenic Potency Database)
- What structures to supply?
 - Synthetic route
 - Intermediates, reactants, side reaction products, and POTENTIAL side reaction products
 - Final API and product
 - Impurity profile peaks that have reached ICH ID threshold
 - POTENTIAL impurities
 - API and drug product degradants
 - Profile peaks that have reached ICH ID threshold
 - POTENTIAL degradants
 - Reactants and intermediate stability (EMEA)
- How to supply them?
 - Potential impurities and degradants
 - Human Expertise
 - *In silico* “expertise”
 - CAMEO (Cambridgesoft)
 - Real impurities and degradants
 - Structural Elucidation of peaks that have reached ID threshold

Aziridines



How to Assign Potential Genotoxicity

- Genotoxicity testing is usually conducted within a strategy rather than as individual tests.
 - ICH (M3, S2A, & S2B) advocates 3 test, if all negative then safe to proceed.
- *In Vitro* Assays (2)
 - AMES Tests - bacterial reverse mutation assay
 - Measures the induction of gene mutation
 - Uses prokaryotic cells, lacking mammalian repair mechanisms, metabolisms, etc.
 - Not suitable for use with antibiotics or topoisomerase inhibitors, etc.
 - *In Vitro* mammalian chromosome aberration test
 - % of cells with Structural chromosomal aberration as measure of genotoxicity
 - *In Vitro* Micronucleus Assay
 - Common alternative to IVMCA test
 - Can determine structural & numerical aberrations
- *In Vivo* Assays (1)
 - Mammalian erythrocyte micronucleus test
 - In vivo form of IVMN
 - Mammalian bone marrow chromosome aberration test
 - In vivo form of IVMCA test
 - Exposure limitations

“Approaches to the Risk Assessment of Genotoxic Carcinogens in Food: A Critical Appraisal: Annex – an introduction to Genetic Toxicology” (Food and Chemical Toxicology 44, Vol. 10 (2006)), O’Brien, et al

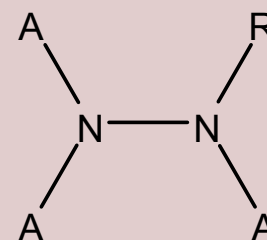
Case Study/ Cautionary Tale

Primary Degradant with Ames Assay Hit

Primary Degradant Qualification

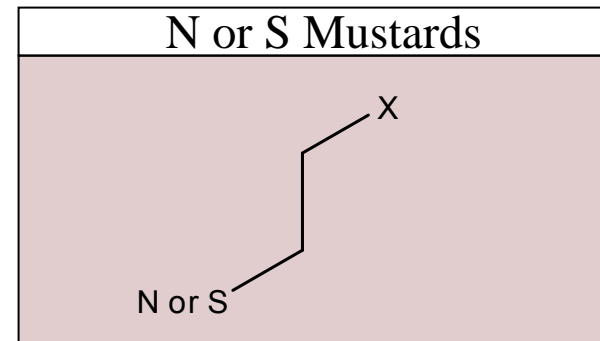
- No alerting structures.
- No suspect data from early tox work
- Qualify above 0.5% level to give formulation FTO as it transitions into full development
 - Impurity is also penultimate intermediate
- *In Vitro* Qualification
 - AMES
 - IVMN and *in vitro* SCA assay
- AMES Hit – VERY unexpected
- What now?
 - Qualify *in vivo* = 6 to 12 month or more delay
 - Treat as genotoxic impurity
 - Cannot fully arrest degradation pathway
 - Would kill the compound

Hydrazines / Azo compounds

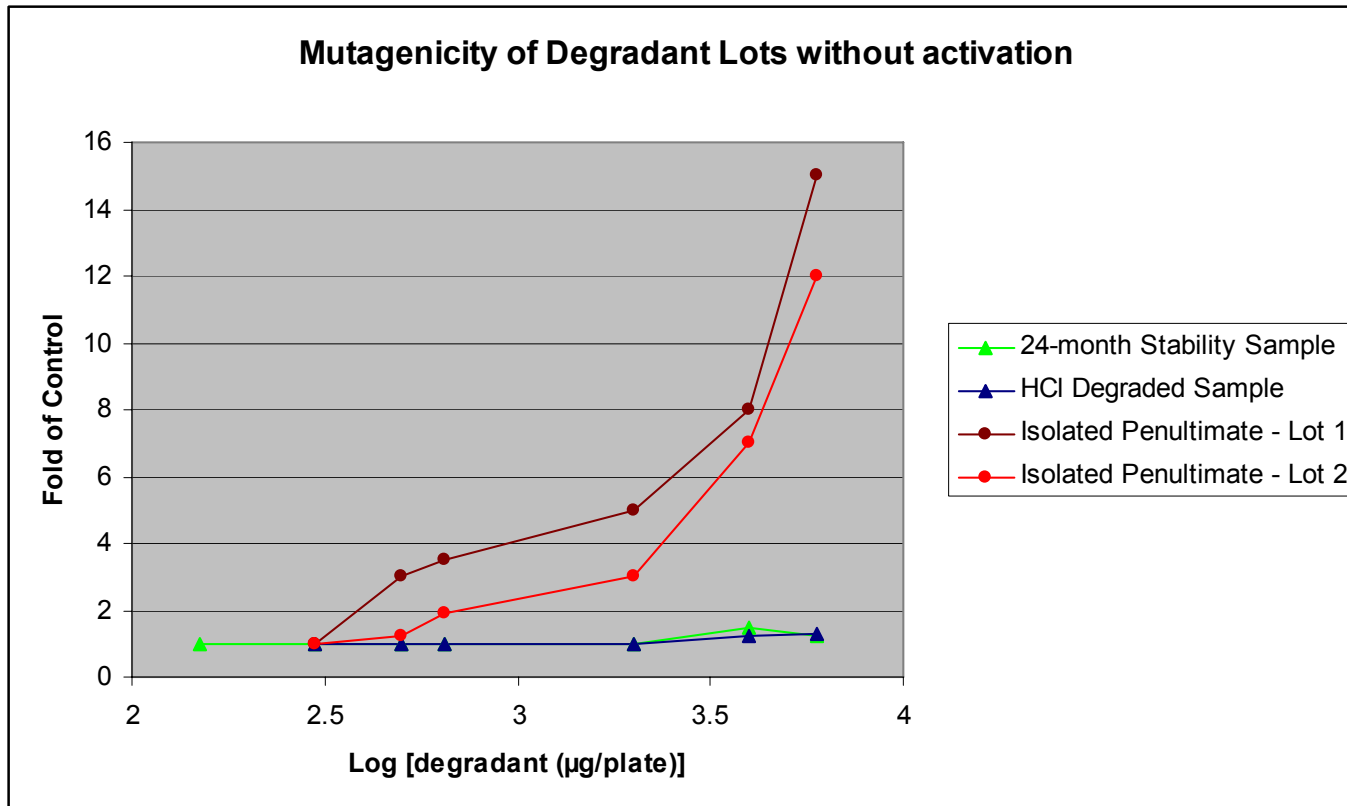


Solutions Oriented Approach

- No longer considering new risks that would be introduced
 - Program timeline already in serious jeopardy
 - Have to isolate the cause or program is dead
- Use AMES test as the analytical “detector”
 - Not ideal, but functional!
- Use multiple sources of impurity as test articles
 - Prototype stability samples
 - Dosed as active enriched in impurity
 - Force degraded samples
 - Isolated and purified by prep chromatography
 - 2 impurity lots from penultimate step of synthesis



It Wasn't the Impurity!!

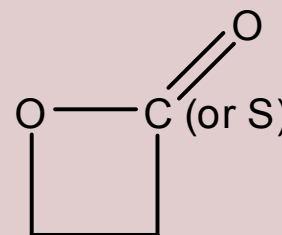


- Results sent us looking for impurity in penultimate step, subsequently Identified process impurity, isolated it, and demonstrated it to purge in final bond forming step

Building from this Experience

- What if we use forced deg samples for up front qualification as in API impurity work
 - Is it representative?
 - Is it better than selectively synthesized substrate?
 - Are there low level ancillary degs produced or excluded?
 - What are the risks of digging in a minefield?
 - Concordance of in vitro tests
 - Purity of samples
 - 1°, 2°, 3° degradants
 - Are these the next frontier of concern?

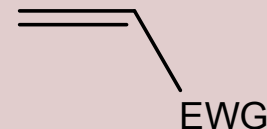
Propiolactones, -sultones



Degradant Points

- Assumption: Degradants virtually always directly related to structure of parent, so PhARMA class 4 = normal control
 - *Not True!!*
- BUT... true degradants most likely will continue to grow under stress
 - So appropriately designed forced deg experiments should elucidate most
 - What about reactive intermediates?
 - Excludes trace contaminant degradation
- Take a 2-pronged Approach
 - Use forced degradation
 - Define degradation “space” and species
 - Use *in silico* predictive tools
 - CAMEO (Cambridgesoft)
- Ames/IVMN “detector” for genotox
 - Tox friends will hate us, but a proven alternative
- Relationship between degradants and metabolites
 - Metabolites may be developed long before development

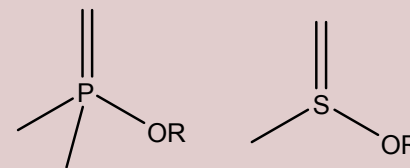
Michael-reactive Acceptors



Impact on the way we do business

- Requires a strong understanding of degradation chemistry by analytical development scientists
- Increasing value of the Analytical Chemist
- Increased value of forced degradation
 - Use forced degradation to identify species
 - Exercise GREAT caution
 - USE RELEVANT REACTANTS
 - Relationship between 1° and 2° degradants
 - Degradation in physiological solutions at physiological T & t
- What if you have a genotoxic degradant?
 - Does it kill your program?
 - Can you eliminate the pathway?
 - Control strategies?
 - Additive: antioxidants, proton scavengers, etc.
 - Eliminative: excipient alternatives, excipient purity, packaging

Alkyl sulphonate esters



Bibliography and Resources

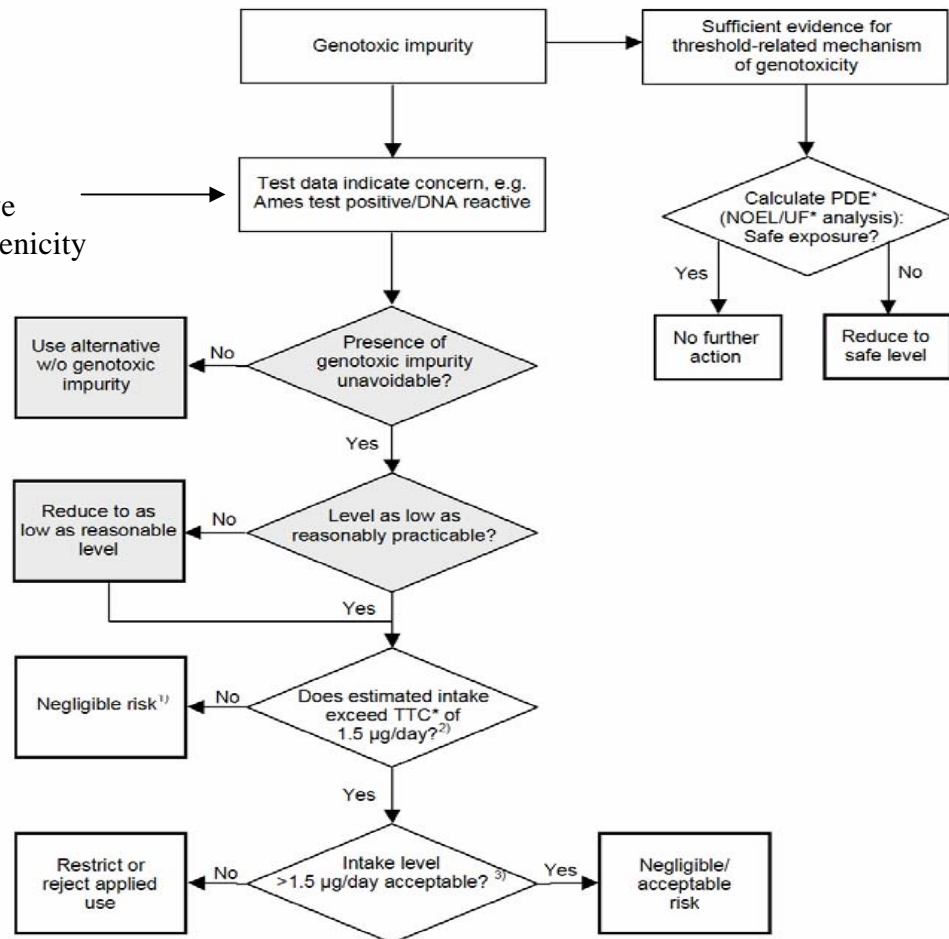
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- Dobo, et al, **The Application of Structure-Based Assessment to Support Safety and Chemistry Diligence to Manage Genotoxic Impurities in Active Pharmaceutical Ingredients During Drug Development.** *Regulatory Toxicology and Pharmacology* (2006), 44, 282-293
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- Jacobson-Kram, D., McGovern, T., **Toxicological Overview of Impurities in Pharmaceutical Products.** *Advanced Drug Delivery* (2007), 59, 38-42
- O'Brien, et al, **Approaches to the Risk Assessment of Genotoxic Carcinogens in Food: A Critical Appraisal: Annex – an introduction to Genetic Toxicology.** *Food and Chemical Toxicology* (2006) 44, Vol. 10
- **National Cancer Institute Lifetime Cancer Risks:** http://surveillance.cancer.gov/statistics/find/lifetime_risk.html

Back-Up slides

EMEA – Decision Tree

If a compound fails an *in vitro* test

- Demonstrate relevance by *in vivo* assay
- If no *in vivo* data, *in vitro* is presumptive of *in vivo* mutagenicity and/or carcinogenicity



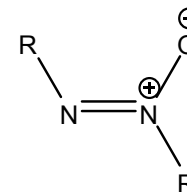
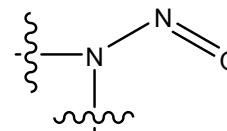
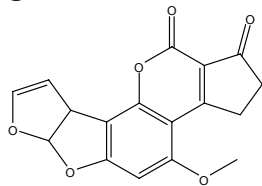
EMEA

- Pharmaceutical Assessment Requirements
 - A specific discussion of genotoxic assessment required in drug application
 - Highlight
 - Chemical process – reagents, intermediates, side-products that are known, suspected, or structurally alerting for genotoxicity
 - Impurity Profile
 - Justification for no alternative (routes, reagents, starting materials,
 - Data on *chemical stability* of reactive intermediates, reactants and other compounds should be included in this assessment.

EMEA – Policy Outliers

□ High Potency Carcinogens Excluded from TTC approach

- aflatoxin-like-
- Nitroso amines
- azoxy-compounds



- Risk assessment of members of such groups requires compound-specific toxicity data.

□ A TTC value higher than 1.5 µg/day may be acceptable under certain conditions

- short-term exposure
- treatment of a life-threatening condition
- when life expectancy is less than 5 years
- where the impurity is a known substance and human exposure will be much greater from other sources (e.g. food).
- Genotoxic impurities that are also significant metabolites may be assessed based on the acceptability of the metabolites.

EMA language around testing of GI's

- limited to those impurities that might reasonably be expected based on knowledge of the chemical reactions and conditions involved. Guided by existing genotoxicity data or the presence of structural alerts, potential genotoxic impurities should be identified. When a potential impurity contains structural alerts, additional genotoxicity testing of the impurity, typically in a bacterial reverse mutation assay, should be considered (Dobo et al. 2006, Müller et al. 2006). While according to the Q3A guideline such studies can usually be conducted on the drug substance containing the impurity to be controlled, studies using isolated impurities are much more appropriate for this purpose and highly recommended.

Issues/Limitations with EMEA

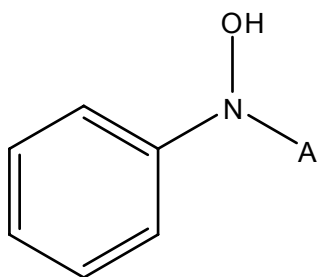
- Applies specifically to Marketed Products
- Acceptable Risk Paradigm Assumptions
 - Assumes Chronic Lifetime (70 yrs) Dosing
 - Focuses on a 0.001% increase in cancer risk
 - Based on 2002 – 2004 cancer rates, 41% of people born today WILL be diagnosed with cancer at some time during their lifetime
(National Cancer Institute)
- “The TTC is a pragmatic risk management tool using a probabilistic methodology, The TTC concept should not be interpreted as providing absolute certainty of no risk.” (EMEA)



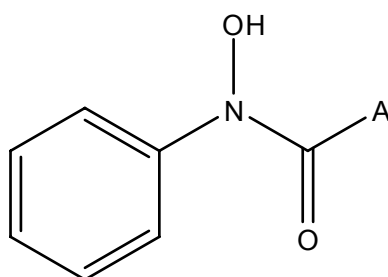
Primary Genotoxic Impurity Concerns

- DNA Adduct Formation – Preventing accurate replication
 - Includes covalent bond formation on the DNA bases
 - Alkylation
 - Cyclization
 - Intercalation
 - Aromatic amines
 - Electrophillic, forming strong covalent bonds with DNA
 - Alkylating agents

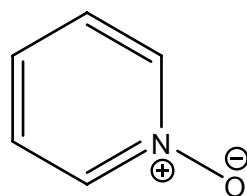
SA Group 1: Aromatics



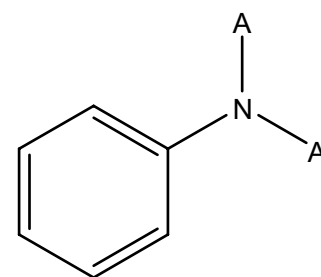
N-Hydroxyaryls



N-Acylated aminoaryls

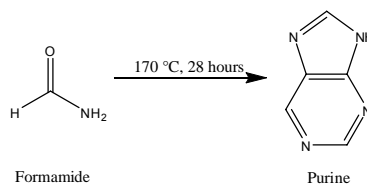


Aza-aryl N-Oxides

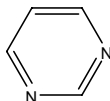


Aminoaryls
and alkylated aminoaryls

- Aniline based molecules
- Purines (think nucleic acids)

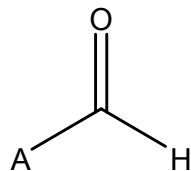


- Pyrimidines (think nucleobases in cytosine, thymine, uracil)

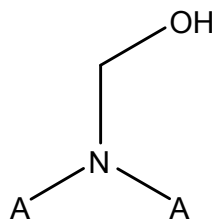


- Intercalators – Bind to DNA and insert into structure

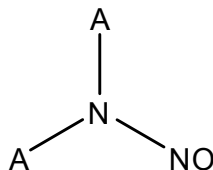
SA Group 2: Alkyl & Aryl Groups



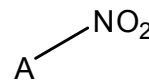
Aldehydes



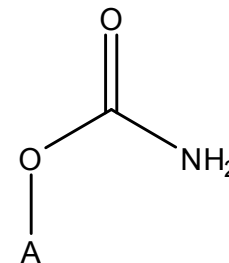
N-Methylols



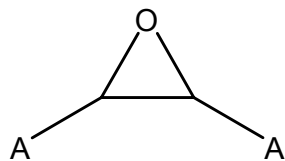
N-Nitrosamines



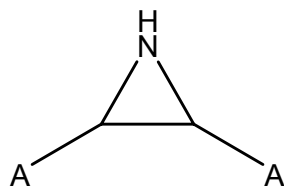
Nitro
compounds



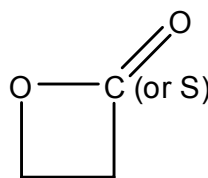
Carbamates (Urethanes)



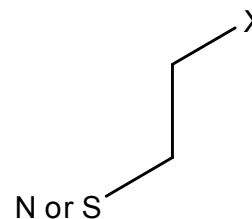
Epoxides



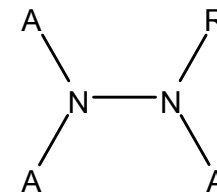
Aziridines



Propiolactones
Propiosultones

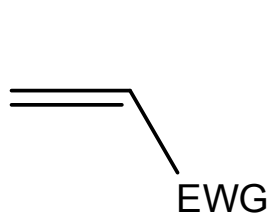


N or S
Mustards

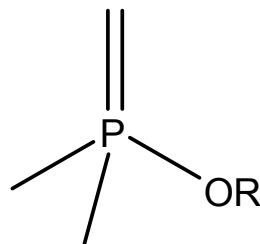


Hydrazines
and
Azo compounds

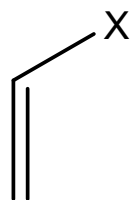
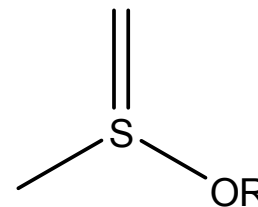
SA Group 3: Heteroatomic Groups



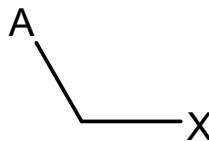
Michael-reactive
Acceptors



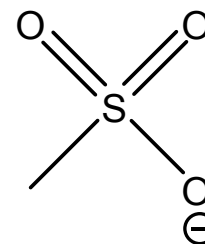
Alkyl Esters of Phosphonates or
Sulfonates



Halo-alkenes



Primary Halides
(Alkyl and aryl-CH₂)



Mesylates