In Silico/In Vitro Test Methods for Genetic Toxicology Assessment of Food Ingredients: Benefits and Challenges

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SOT Food Safety Specialty Section

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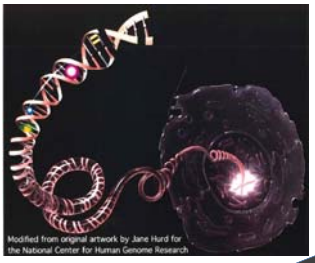
Agenda

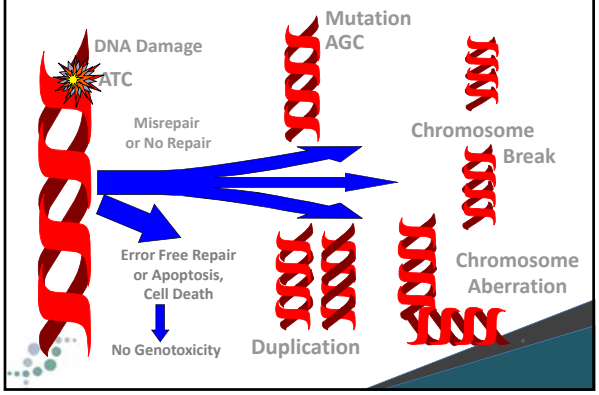
- **What is Genetic Toxicology?**
- **Regulatory Guidelines/Approaches for Food Ingredients**
- ***In Vitro*/*In Silico* Approaches for Genotoxicity Assessments**

What is Genetic Toxicology?

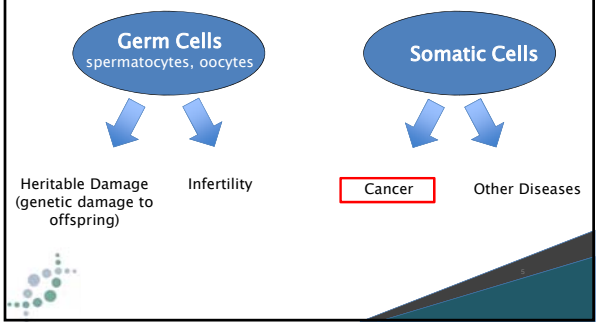
… the study of chemical, physical or biological agents that can change the sequence or structure of DNA



What is Genetic Toxicology?



Adverse Effects Caused By Genotoxicity



Goal of Genotoxicity Testing Food Ingredients

- Food manufacturers want products to be safe and perceived as safe
- Need to meet global regulatory requirements

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Regulatory Approaches For Food Substances

- United States
  - Generally recognized as safe (GRAS)
  - FDA Center for Food Safety and Applied Nutrition (CFSAN) Food additive petition; Food contact notification, follows Redbook
    www.fda.gov/AboutFDA/CentersOffices/OfficeofFoods/CFSAN/
- Europe: European Food Safety Authority (EFSA) www.efsa.europa.eu

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US FDA CFSAN

- Food additives (2006)
  http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm054658.htm
- Food Contact Notification Recommendation (2002)http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm081825.htm
- Draft Dietary Supplements (Aug 2016)
  http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/dietarysupplements/ucm257563.htm

European Food Safety Authority (EFSA)

- **EFSA** is the European Union risk assessment body for food and feed safety
  - independent scientific committees provide advice to risk managers
- Scientific committee opinion on genotoxicity testing strategies applicable to food and feed safety assessment; EFSA Journal 2011;9(9):2379
  - EFSA 2016 Jan 28, 2016 Opinion on food contact

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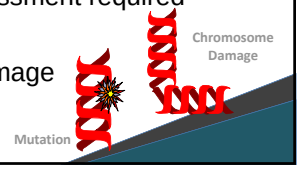
EFSA Genotoxicity Testing Approach

- Tiered Testing Battery
  - Initial *in vitro* tests
  - Follow-up *in vivo* tests
- Multi-endpoint assessment required
  - Mutation
  - Chromosome damage



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EFSA Genotoxicity Testing Approach: Initial Tests

- Bacterial reverse mutation assay "Ames assay" (OECD TG 471), **and**
- *In* vitro assay for chromosome damage (micronucleus test, OECD TG 487)

"Addition of any further *in vitro* mammalian cell tests in the basic battery would significantly reduce specificity with no substantial gain in sensitivity"

Negative-no further testing

Positive, equivocal
- •Evaluate data on ingredient and structurally related
- •Repeat *in vitro* tests
- •Conduct in *in vivo* tests

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EFSA Genotoxicity Testing Approach

- "EFSA scientific committee considered whether an *in vivo* test should be included in the first step of testing and broadly agreed that it should not be routinely included" EFSA Journal 2011;9(9):2379
- Exceptions:
  - Specific metabolism lacking *in vitro*
  - *In vitro* test system inappropriate
  - If repeat-dose *in vivo* studies will be conducted anyway, consider incorporation of genotoxicity

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In Vivo Tests to Follow-up Positive Results *In Vitro*

- Mammalian erythrocyte micronucleus test (OECD TG474)
  "Broad experience with the micronucleus test shows feasibility of integrating both blood and bone marrow micronucleus analysis into repeat dose studies"
- Transgenic rodent somatic and germ cell gene mutation assay (OECD TG 488)
- *In vivo* Comet assay (OECD TG489)
  "Due to its flexibility, the *in vivo* Comet assay could easily be incorporated into repeat dose studies"

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Food Contact/Additive : US FDA, CFSAN

Testing needed when cumulative intake is > 50 ppb in diet (150 ug/person/day)

If intake is 0.5-50 ppb, then only *in vitro* assays (a and b) needed

a. *In vitro* gene mutation in bacteria (Ames test)

b. *In vitro* cytogenetic test for chromosome damage using mammalian cells OR *In vitro* mammalian cell gene mutation assay (mouse lymphoma thymidine kinase +/- preferred)

AND

c. *In vivo* test for chromosome damage in bone marrow (Micronucleus assay)

Regulations listed in Redbook 2000 (updated 2007)

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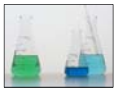
A Standard Genetic Toxicology Testing Approach (1970's)

In Vitro Genotoxicity Assays → Positive Results → *In Vivo Genotoxicity Assays*



Mice, rats

Benefits
- Inexpensive
- Quick
- Detects most genotoxins
- Guarantee cellular exposure to the test article
- Reduce animal use consistent with 3R's

Benefits
- Normal metabolic activation/detoxification
- Normal tissues

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Changes To Genetic Toxicology Assessment

- Increased emphasis on faster, non-animal methods
  - 3Rs
    - *Replacement - use of non-animal methods*
    - *Reduction - methods which reduce the number of animals used*
    - *Refinement - methods which improve animal welfare*
  - *In vivo* assays impractical with large scale testing programs
    - REACH
    - ToxCast
- Examples of inclusion 3R principles in recent guidelines
  - OECD genotoxicity guidelines updated 2013-2015
  - ICH M7 drug impurities —use of *in silico* QSAR 2014

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Current Genetic Toxicology Assessment Approaches

In Silico Structure Activity Relationship (QSAR) Assessment

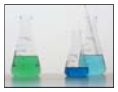
Benefits:
•No need to synthesize chemical
•Inexpensive
•Fast

Challenge:
•Insufficient data to make prediction

Screening *In Vitro* Genotoxicity Assays

Benefits:
•Less test chemical needed
•Inexpensive
•Fast

Regulatory *In Vitro Genotoxicity Assays*



Regulatory *In Vivo Genotoxicity Assays*

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Challenges: *In Vitro* Genotoxicity Assays

Current In Vitro Genotoxicity Tests Have High Frequency of "False Positive" Results

- 75% to 90% rodent non-carcinogens are positive in one or more *in vitro* genotoxicity assays: "false positive" "misleading positive"
  - David Kirkland, Marilyn Aardema, Leigh Henderson, Lutz Müller (2005) Mut. Res 584
  - David Kirkland, Marilyn Aardema, and Lutz Müller (2006) Mut. Res. 608
  - Matthews *et al.* Reg Tox Pharm 44 (2006a; 2006b)

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Challenges: Reasons for False Positive Results *In Vitro*

- *In vitro* genotoxicity assays were designed to "not miss anything" since *in vivo* assays would be used for follow-up testing for biological relevance
  - Use of p53 deficient cells in many assays
  - DNA repair deficient cells
  - High, physiologically irrelevant concentrations/conditions
  - Assays require addition of exogenous metabolic activation system (e.g. arochlor-induced rat liver S9) emphasizes activation, not detoxification
  - Tweats et al Mutagenesis 22, 5-13, 2007

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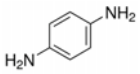
Example: Exogenous rat liver S9 metabolic activation vs normal metabolism



P-phenylenediamine

- Almost all aromatic amines (aa) are genotoxic *in vitro* in the presence of rat liver S9 metabolic activation
- Aromatic amines can be N-acetylated (detoxification) *in vivo*, including in humans (Nohynek et al, *Food Chem Toxicol*, **42**, 1885-1891) to non-genotoxic species
- Rat liver S9 no/minimal N-acetylation activity and emphasizes activation reactions to genotoxic metabolites

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Solutions

- Improve *in vitro* genotoxicity assays
  - Use p53 competent human cells
  - Test more physiologically relevant concentrations
    - 10 mM vs 1 mM (drugs only)
  - Modify assays for specific ingredients (Treat/plate Ames assay for ingredients containing *his* or *try* amino acids)
- Development of 3D tissue models for genotoxicity testing
  - More biologically relevant vs *in vitro* cell cultures

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Conclusions

- Many *in vitro* and *in silico* approaches available for evaluating genotoxicity
- Decades of experience with *in vitro* genotoxicity assays
  - Benefits and limitations are known
- Genotoxicity guidelines are moving towards in vitro approaches
  - Negative results in vitro considered sufficient
  - Positive results require in vivo testing to determine biological relevance
- Development of more predictive approaches is underway

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Thank You for Your Attention!

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