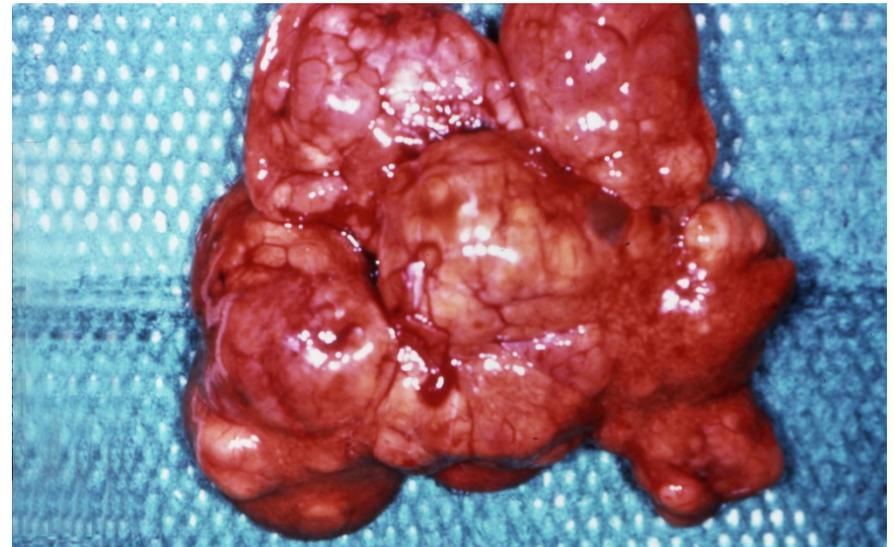
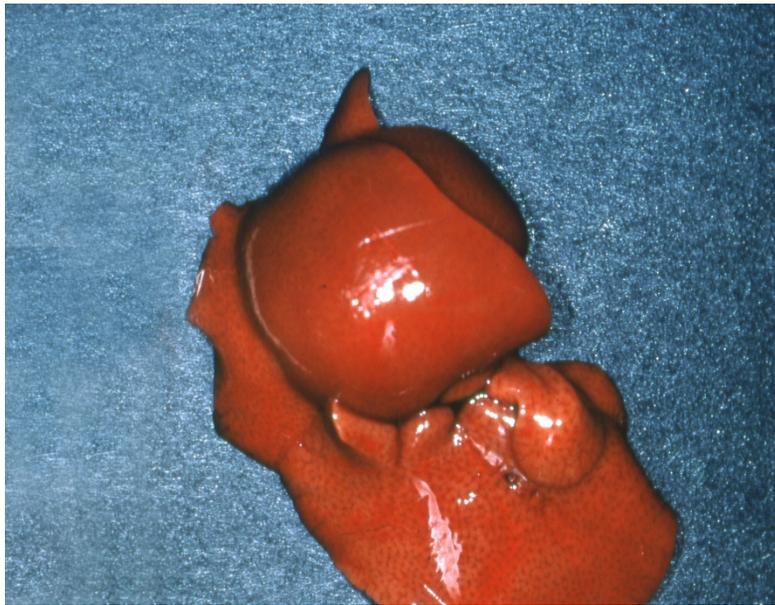
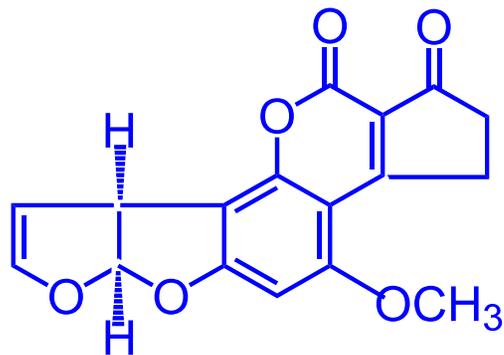


# Use of Chemical Genetics to Dissect The Complex Pathway of Aflatoxin B<sub>1</sub> Carcinogenesis



NIEHS-Superfund  
Meeting

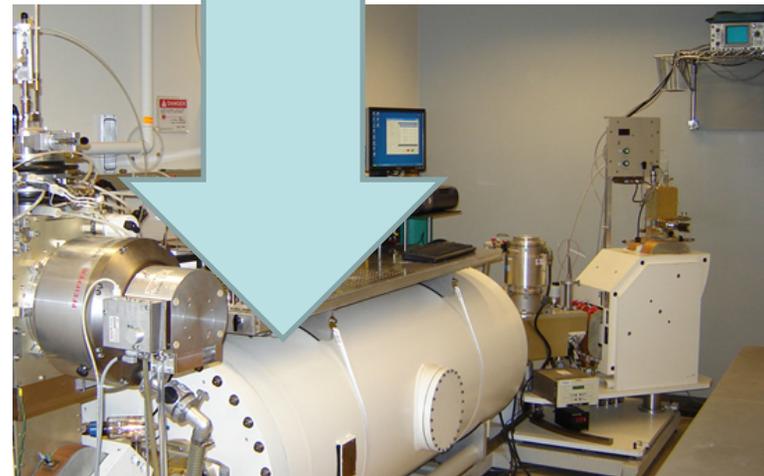
John M. Essigmann



Department of  
Chemistry, Department  
of Biological Engineering  
and Center for  
Environmental Health  
Sciences, MIT

# B6C3F1 Mouse as a Regulatory Model

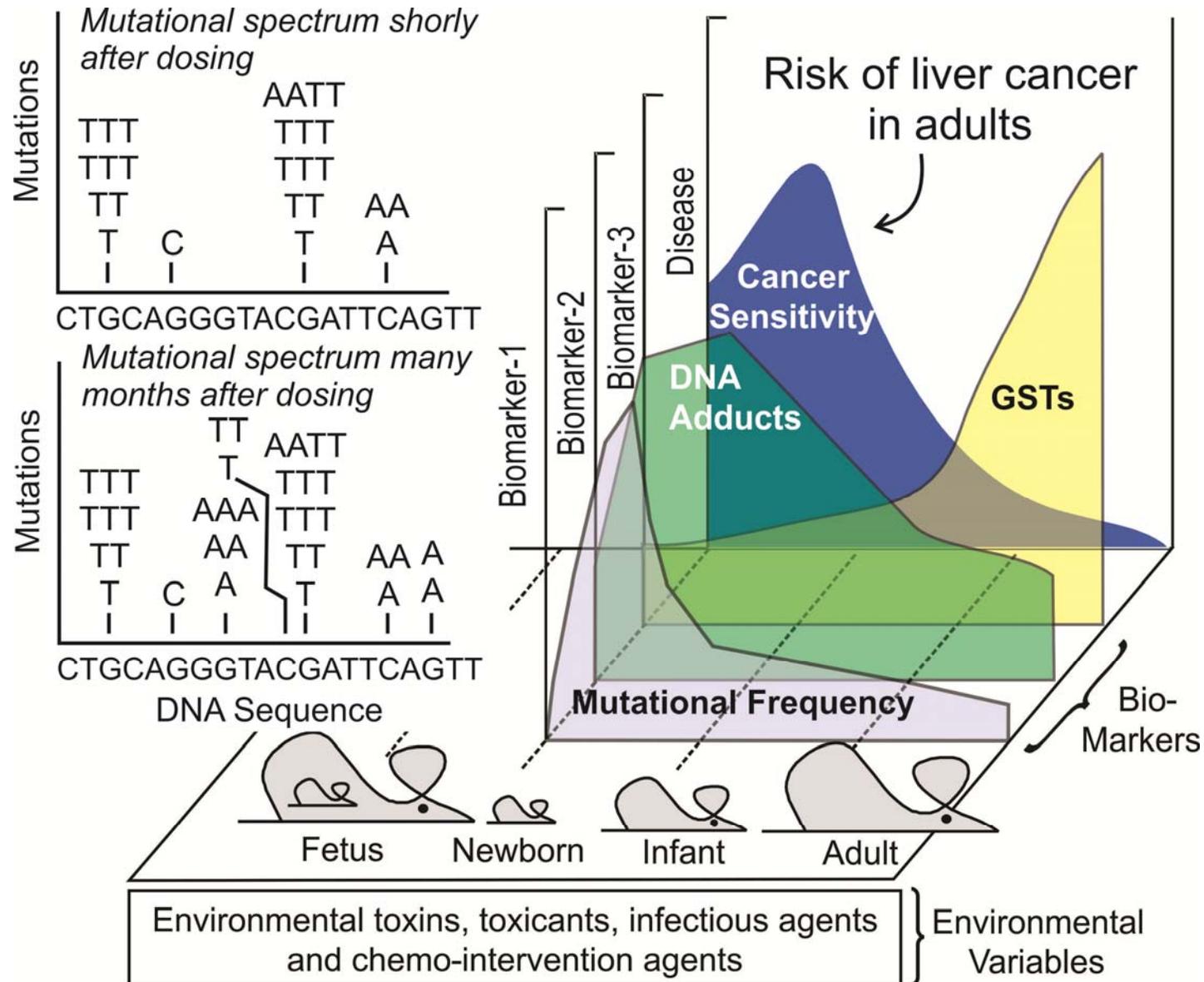
- Always controversial
- Rather than scrap it,
  - Upgrade the mouse (genetically, pharmacologically)
  - Use more modern tools to probe its response to simple (p.o.; i.v., etc.) and complex (e.g., in utero) exposures
  - Make exposure more realistic (concomitant with inflammation, etc.)



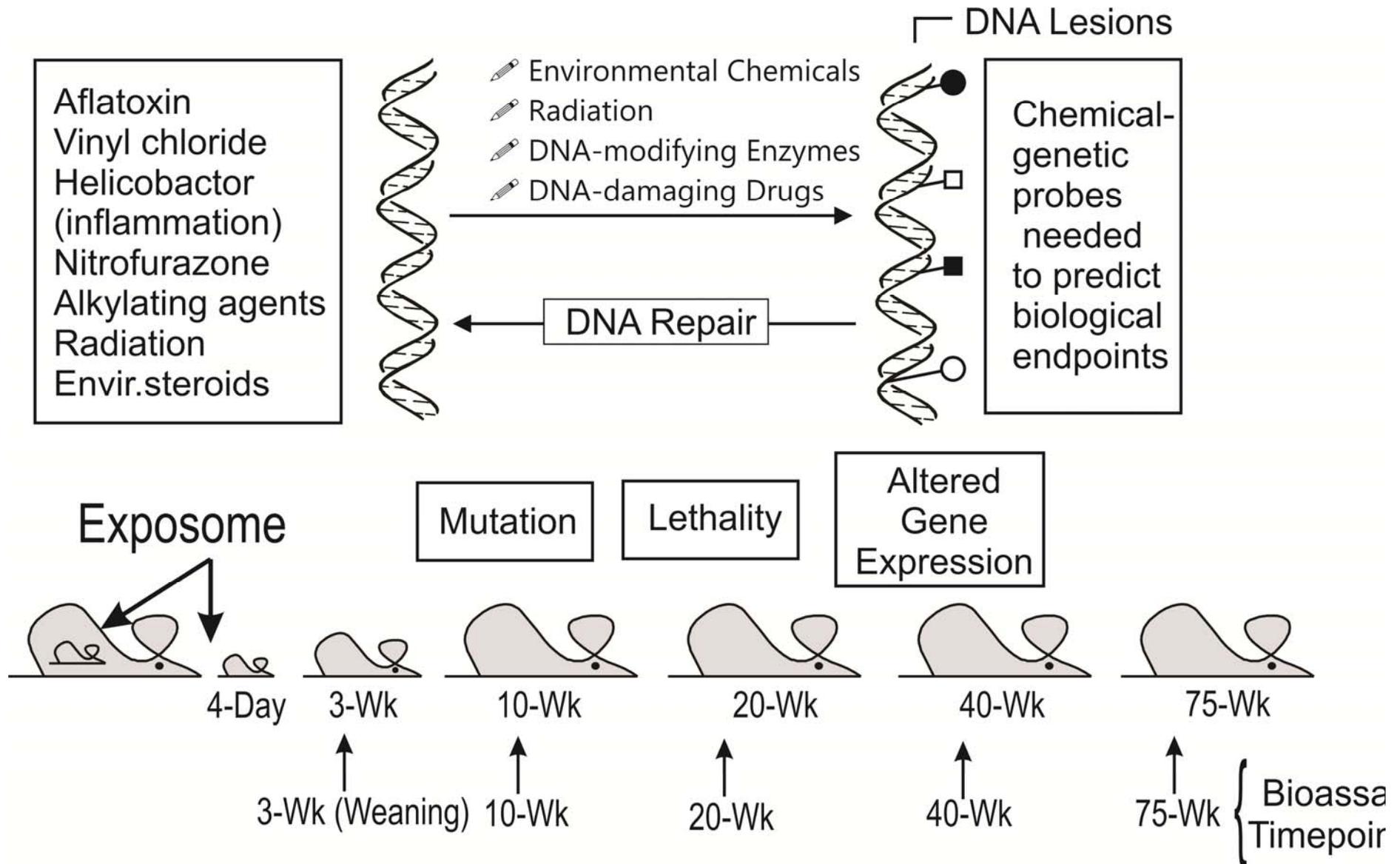
See: A. P. King-Herbert, R. C. Sills and J. R. Bucher, *Tox. Pathol.* (2010)

# Do a Deep Dive on the B6C3F1

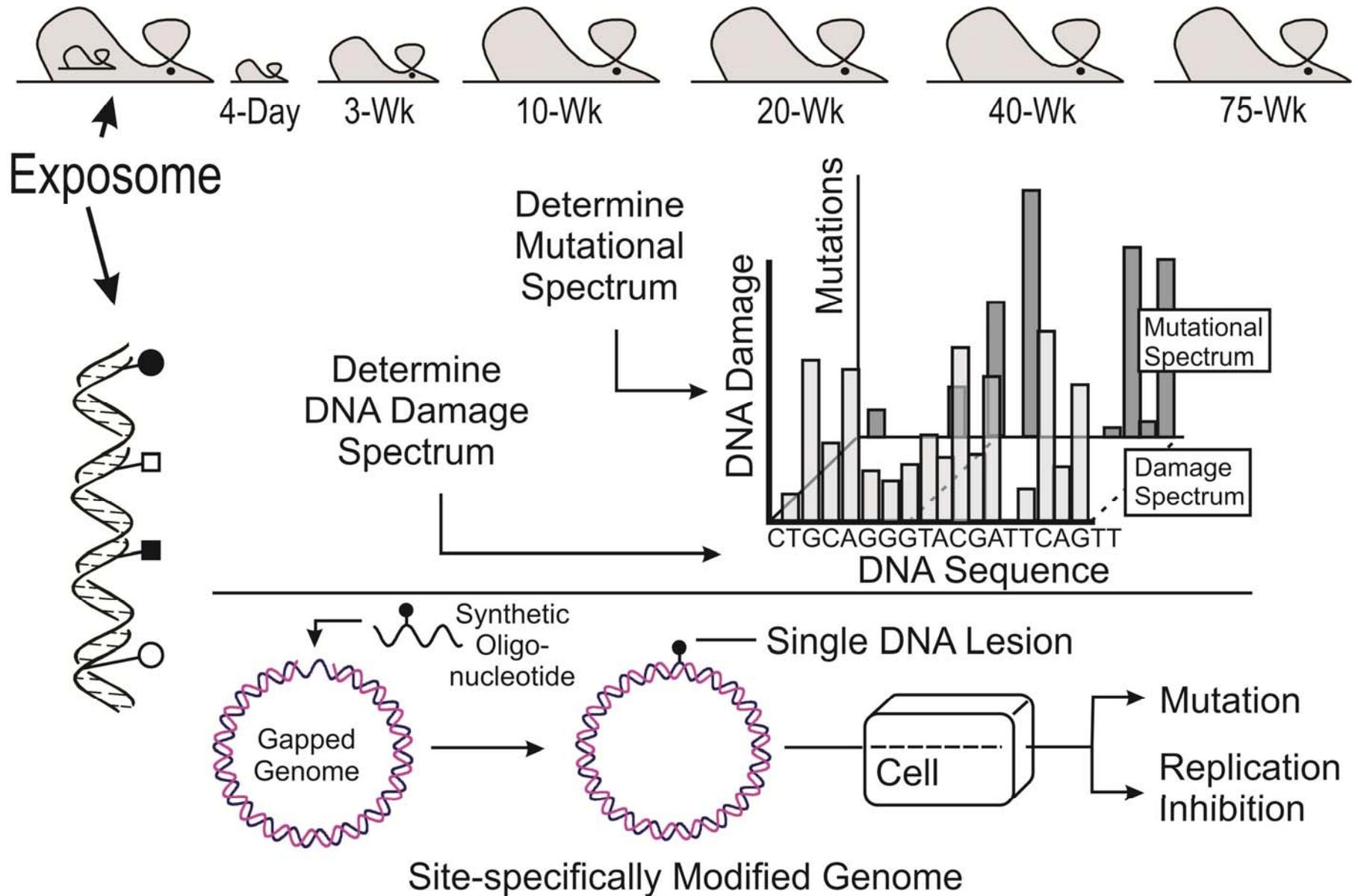
*Use modern tools to mine it for all the data we can get*



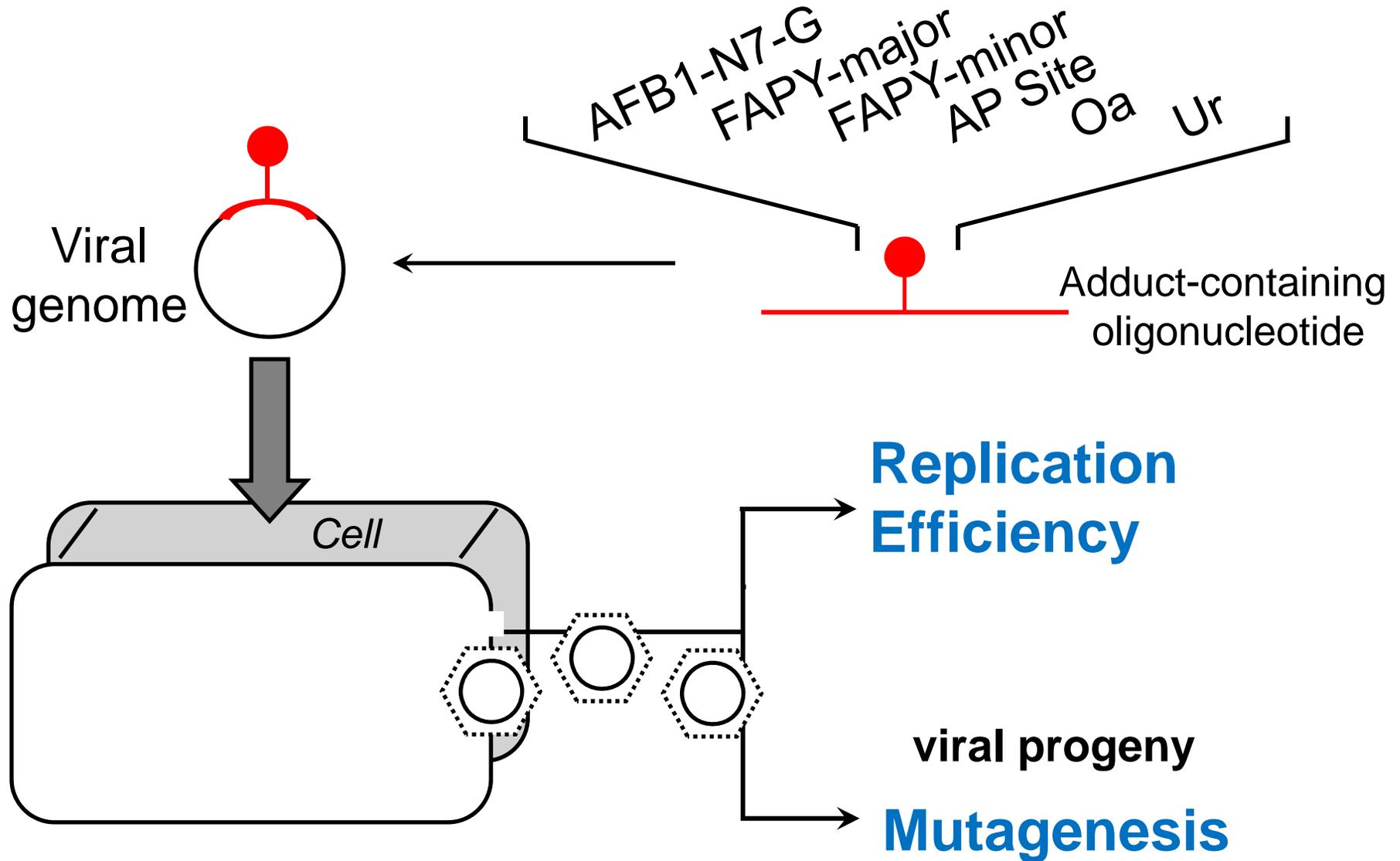
# The Exposome Influences Disease Risk: Biomarkers needed



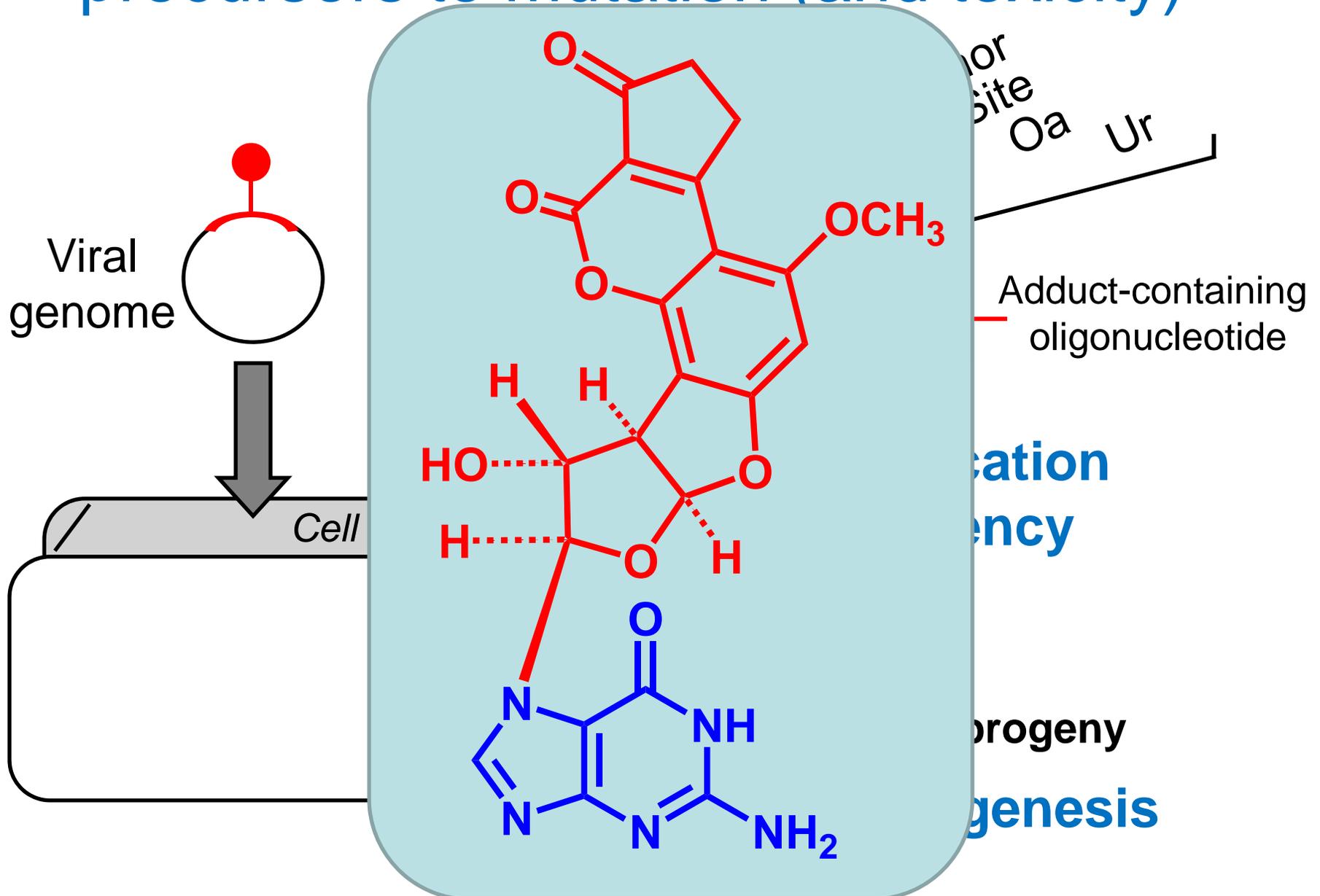
If we want to use DNA adducts as biomarkers, which ones are the important drivers of disease?



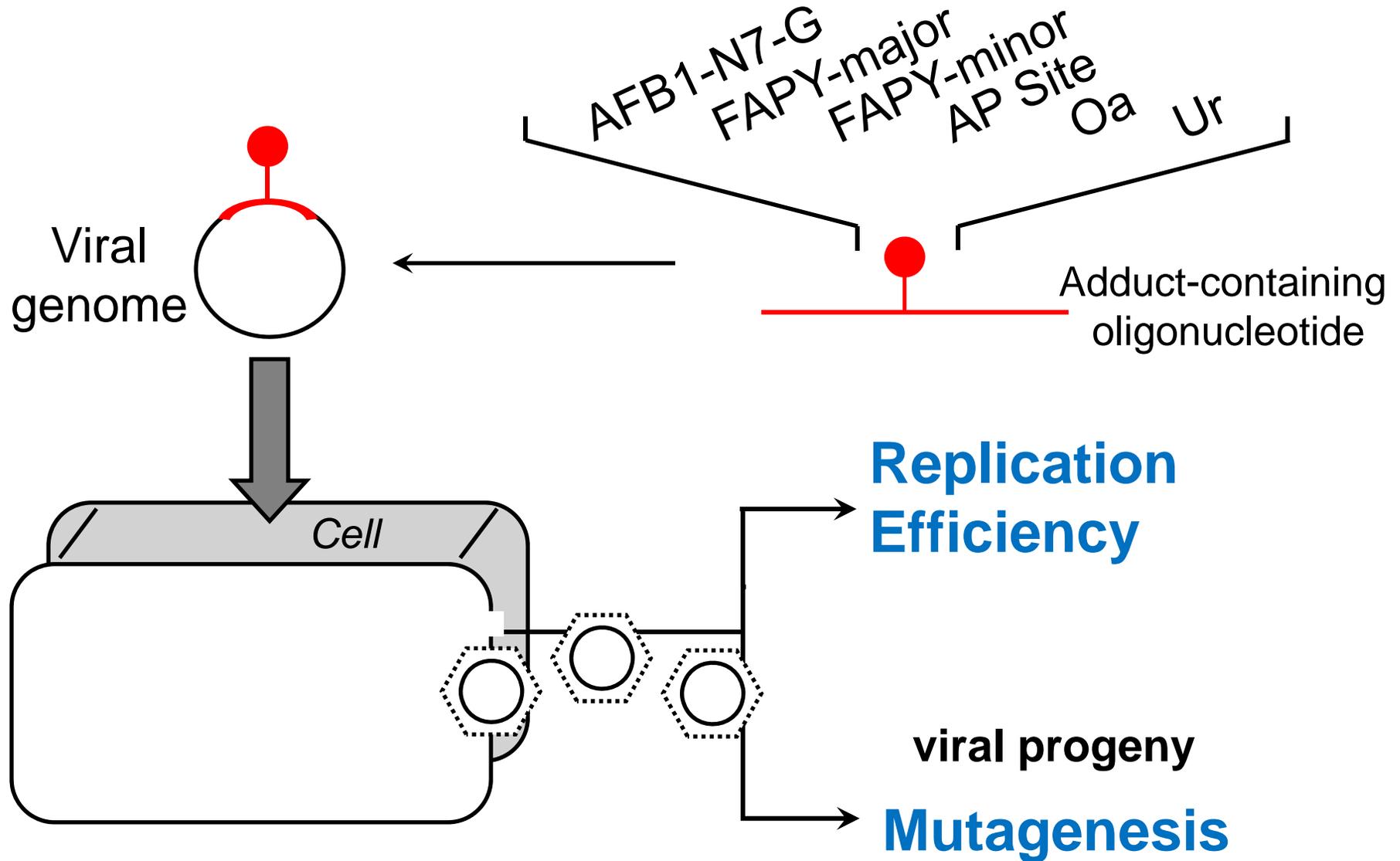
# Identifying AFB1 adducts as the precursors to mutation (and toxicity)



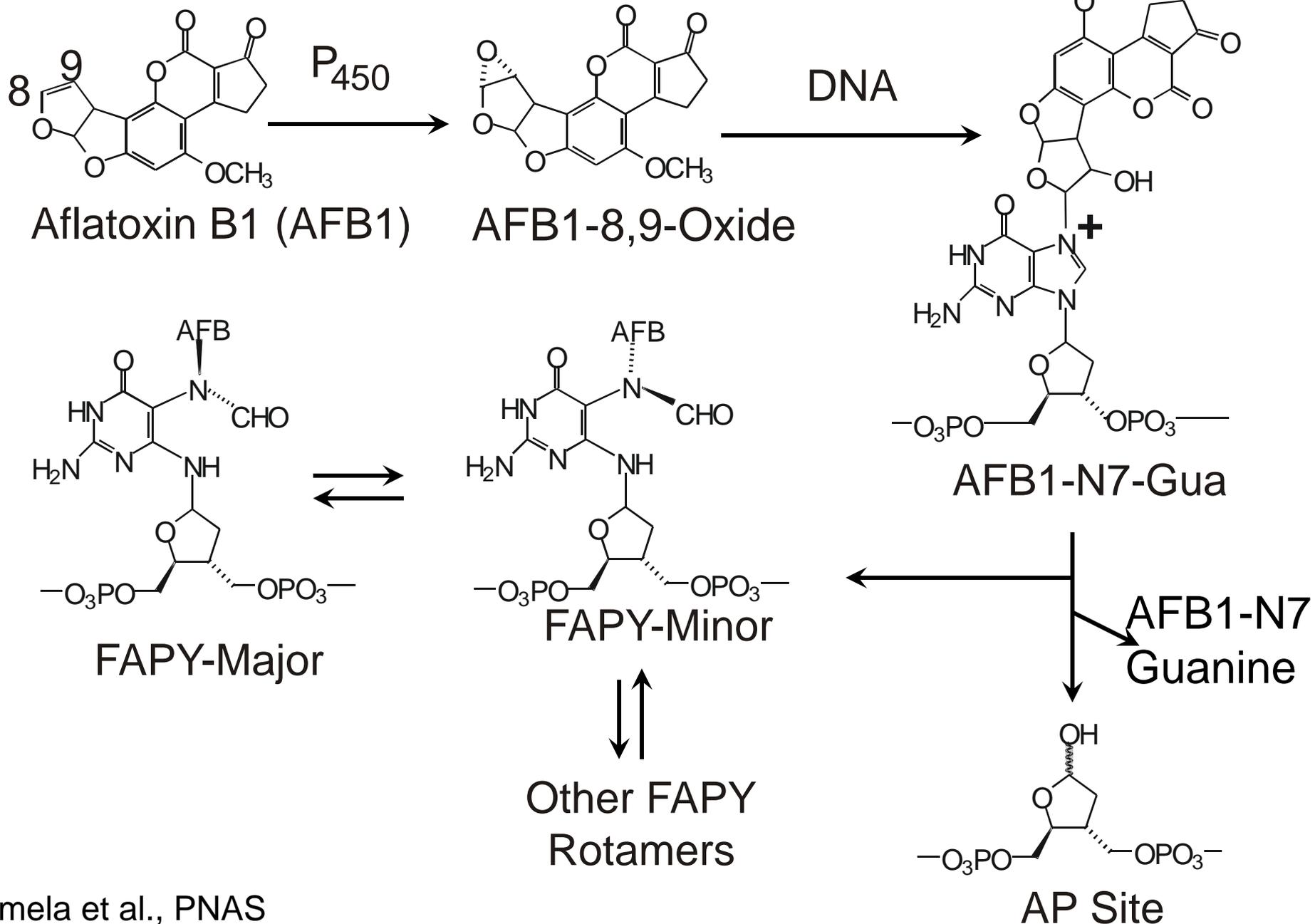
# Identifying AFB1 adducts as the precursors to mutation (and toxicity)



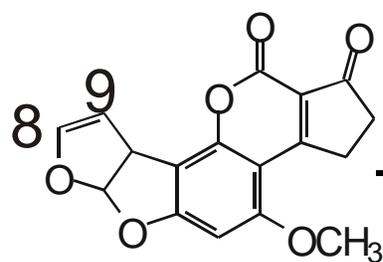
# Identifying AFB1 adducts as the precursors to mutation (and toxicity)



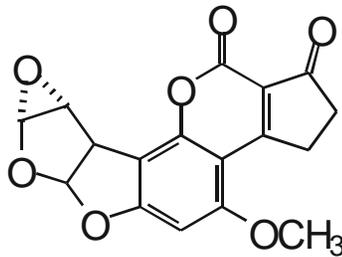
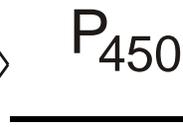
# Search for the Most Mutagenic Lesion



# Search for the Most Mutagenic Lesion

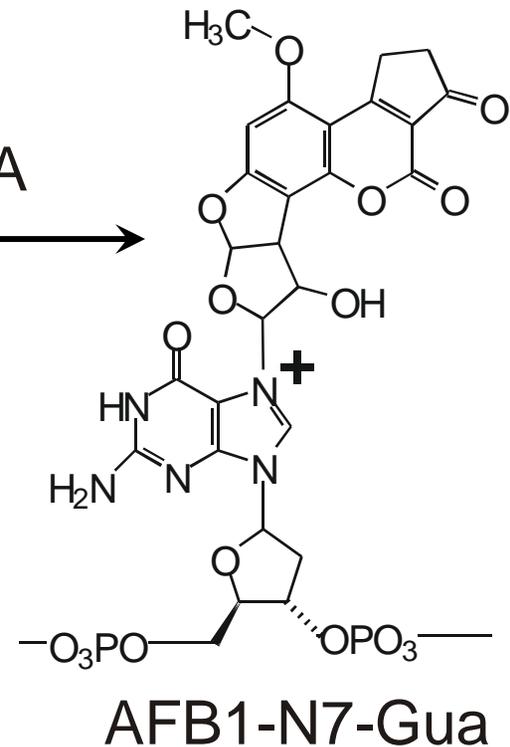


Aflatoxin B1 (AFB1)

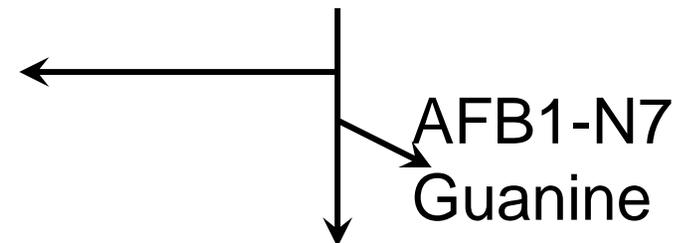


AFB1-8,9-Oxide

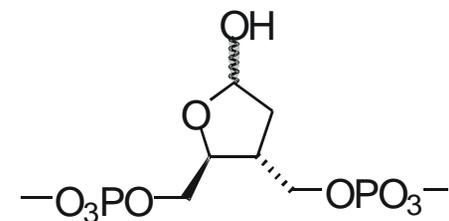
DNA



AFB1-N7-Gua

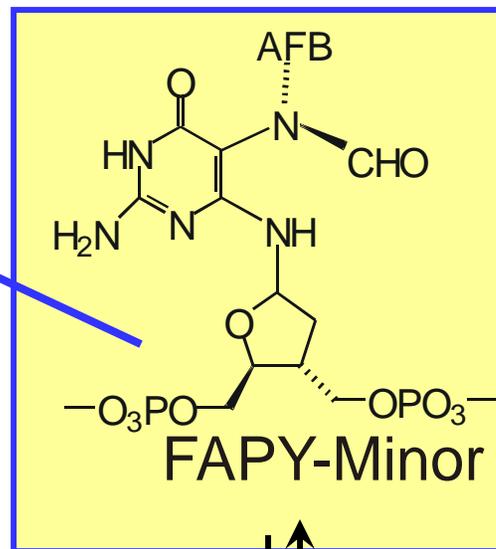


AFB1-N7-Guanine



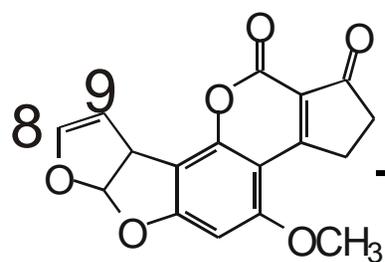
AP Site

- Abundant
- Strongly mutagenic (35%)
- Causes G to T
- Accumulates in liver
- Toxic, but not too toxic
- 1° Candidate as carcinogenic lesion

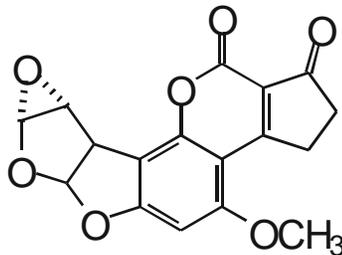
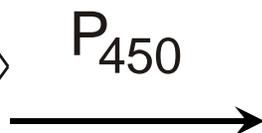


Other FAPY Rotamers

# Search for the Most Toxic lesion

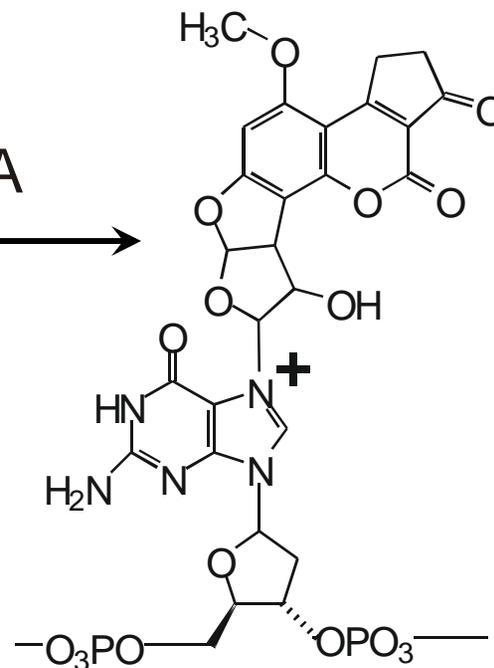


Aflatoxin B1 (AFB1)

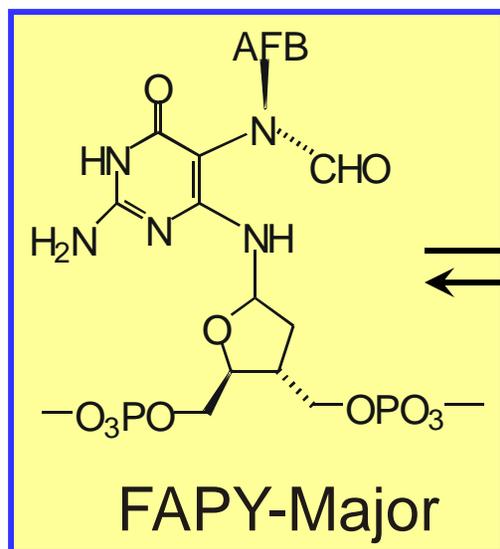


AFB1-8,9-Oxide

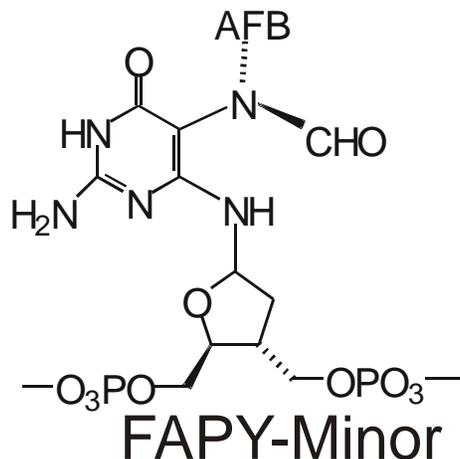
DNA



AFB1-N7-Gua

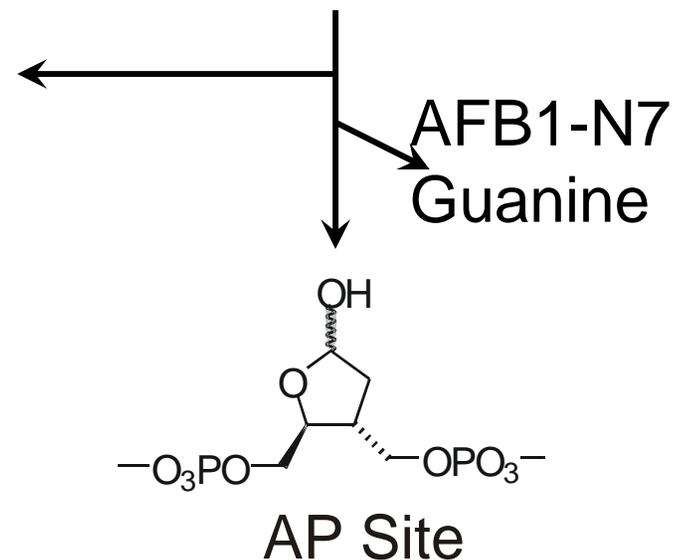


FAPY-Major



FAPY-Minor

Other FAPY Rotamers

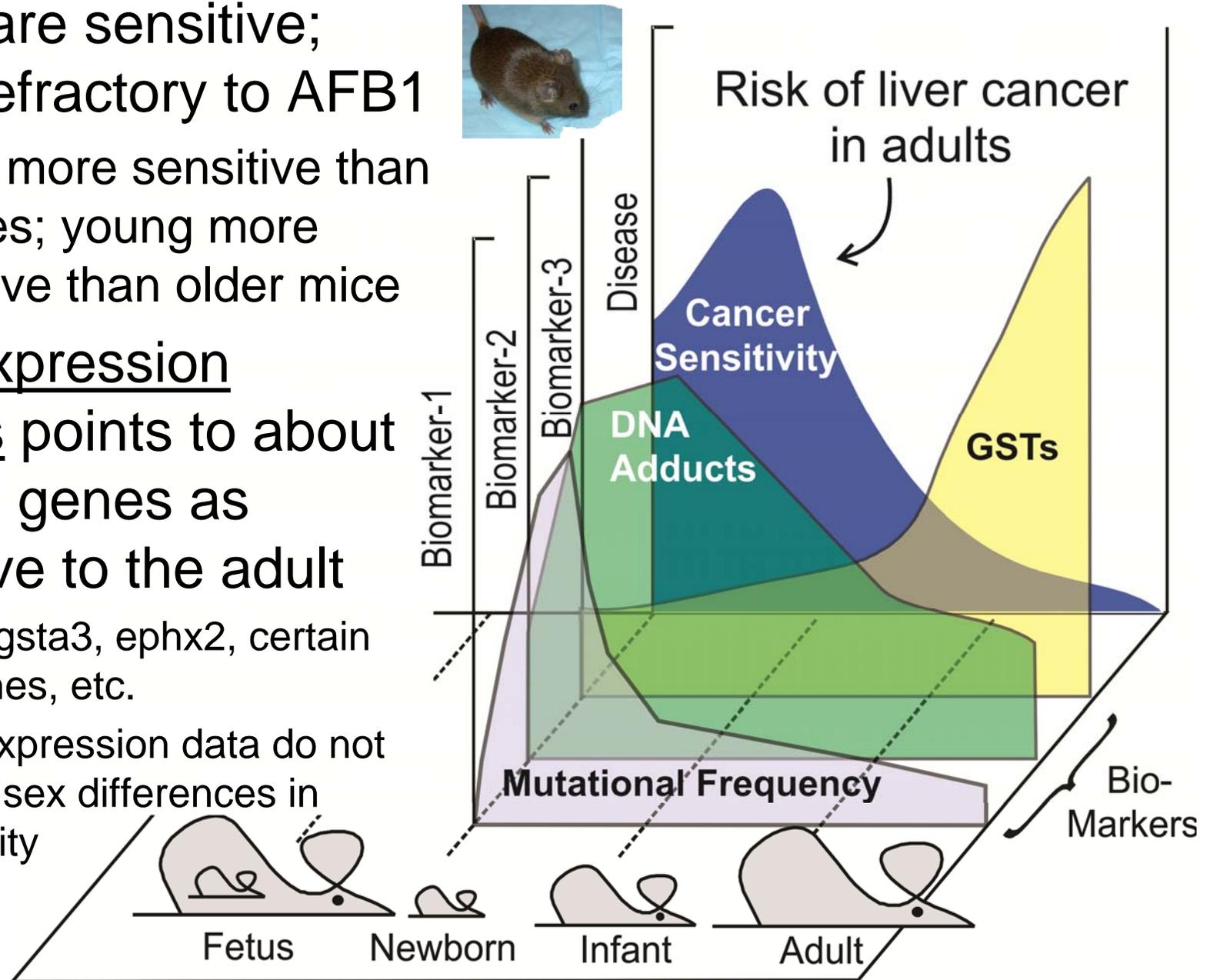


AP Site

- Abundant
- Strongly toxic
- Accumulates in liver
- Principal toxic lesion

# Back to B6C3 F1 mouse model

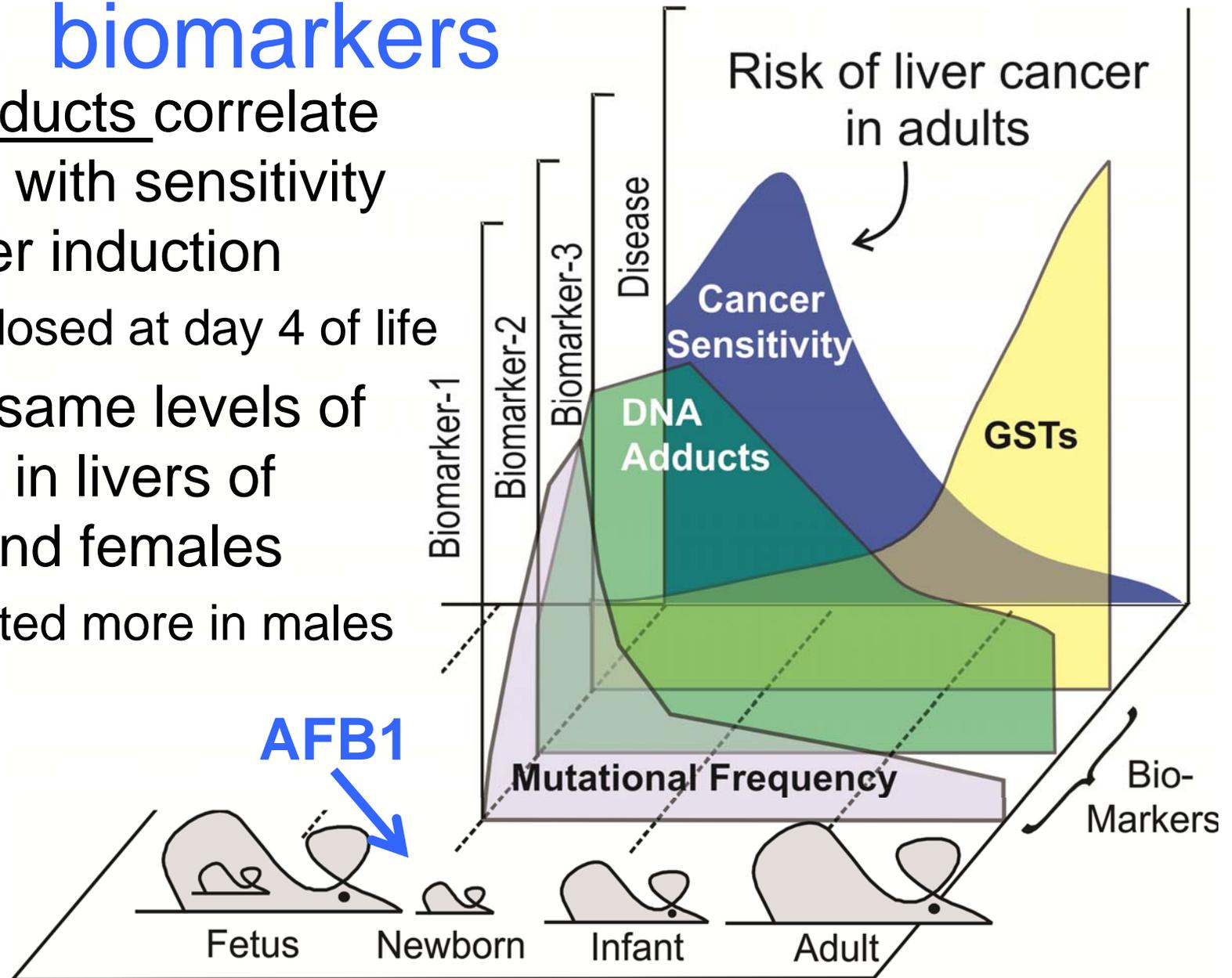
- Infants are sensitive; adults refractory to AFB1
  - Males more sensitive than females; young more sensitive than older mice
- Gene expression analysis points to about a dozen genes as protective to the adult
  - Gsta2, gsta3, ephx2, certain cyp genes, etc.
  - Gene expression data do not explain sex differences in sensitivity





# DNA adducts are useful biomarkers

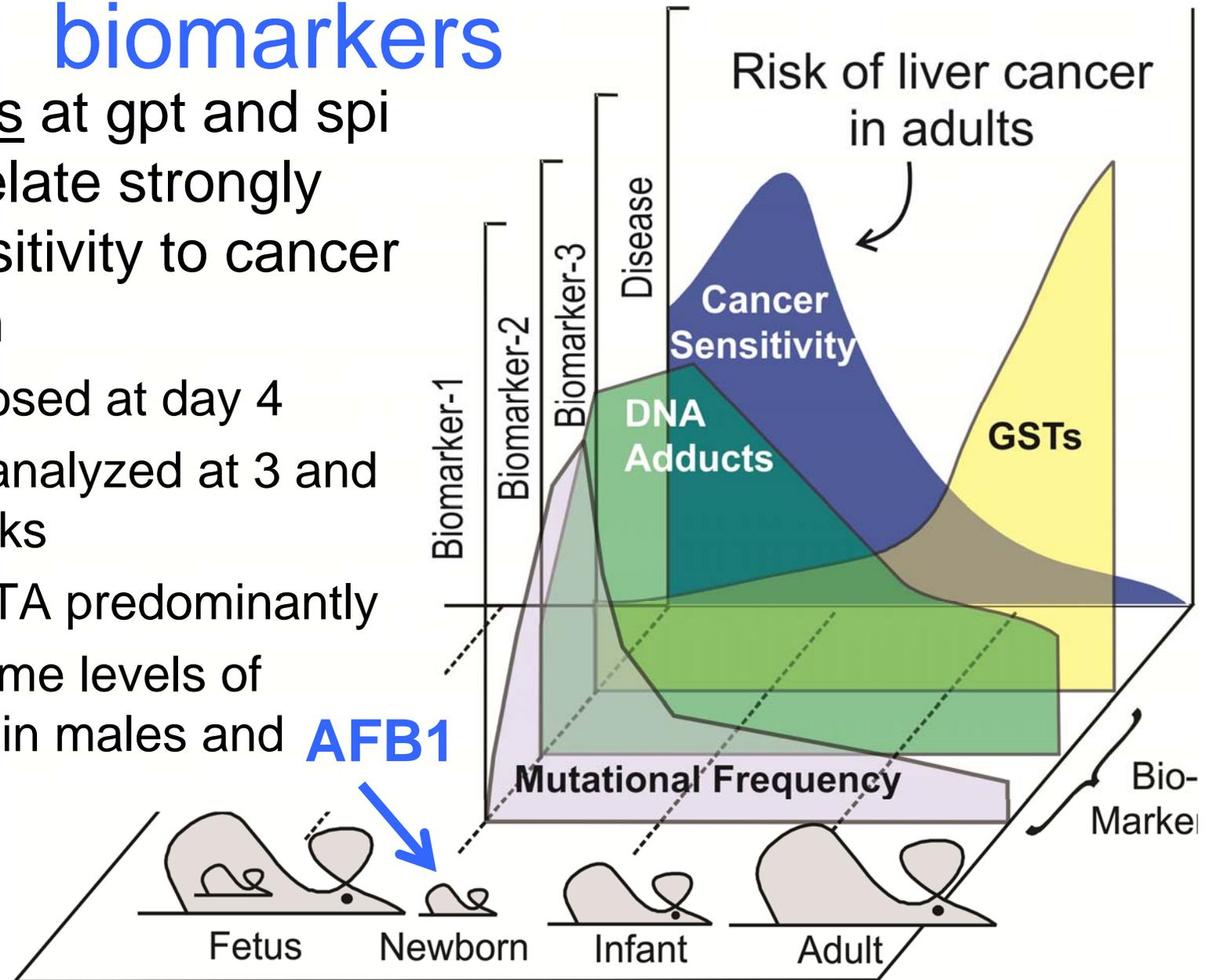
- DNA adducts correlate strongly with sensitivity to cancer induction
  - Mice dosed at day 4 of life
- But the same levels of adducts in livers of males and females
  - Expected more in males





# Mutations are useful biomarkers

- Mutations at gpt and spi loci correlate strongly with sensitivity to cancer induction
  - Mice dosed at day 4
  - Livers analyzed at 3 and 10 weeks
  - GC → TA predominantly
- But the same levels of mutations in males and females

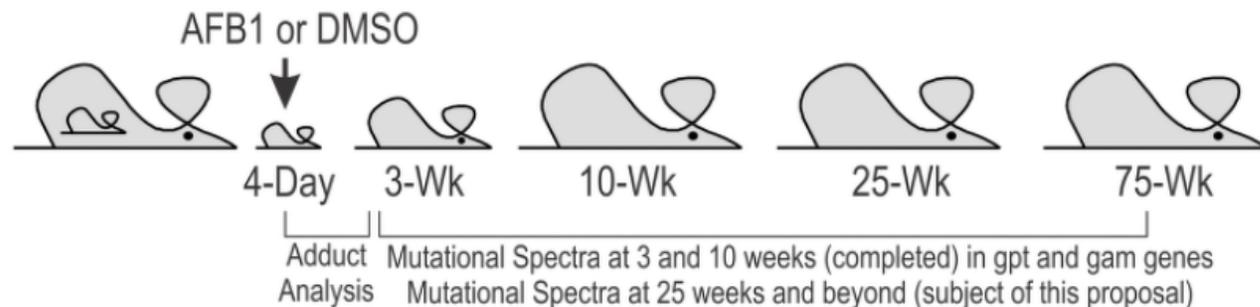
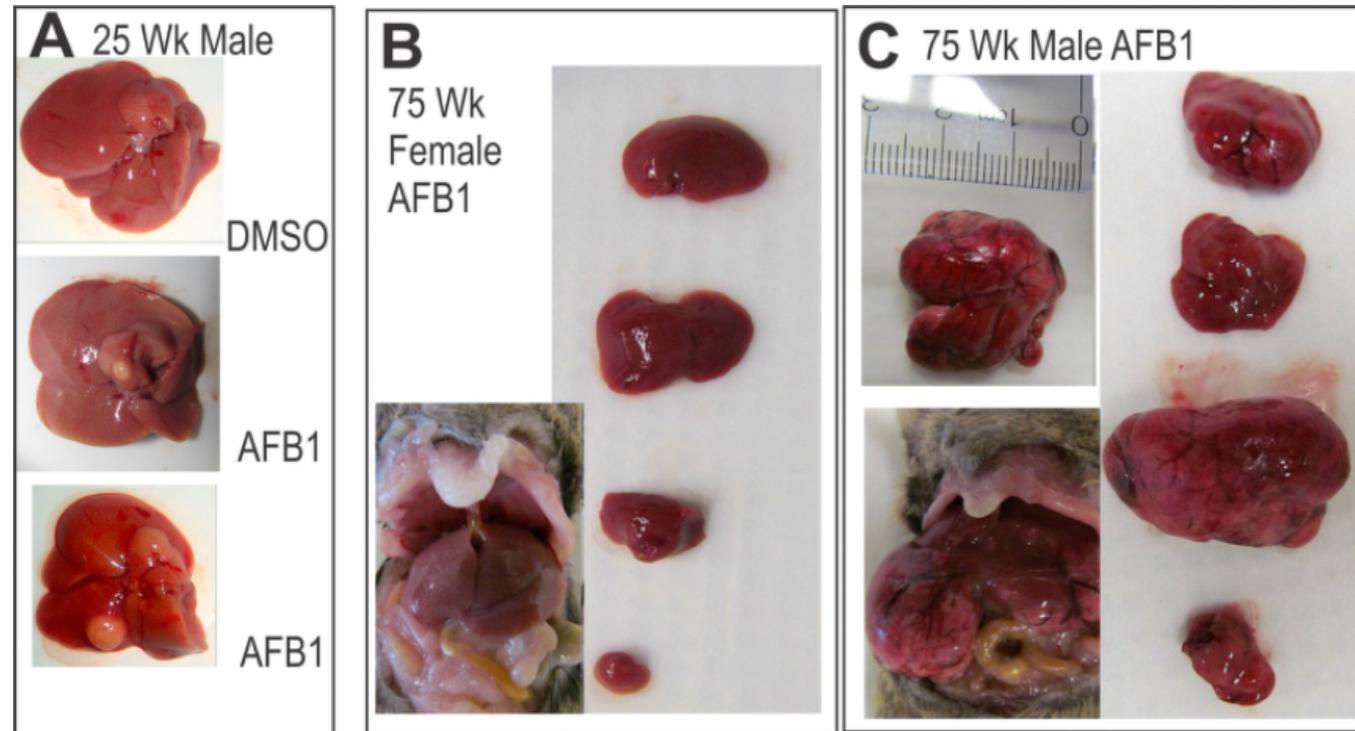


# Mutagenesis: the “right” mutations are there but we fail to see the expected gender differences

## Conclusion:

Continue the experiment to longer time points (beyond the initial 10 weeks)

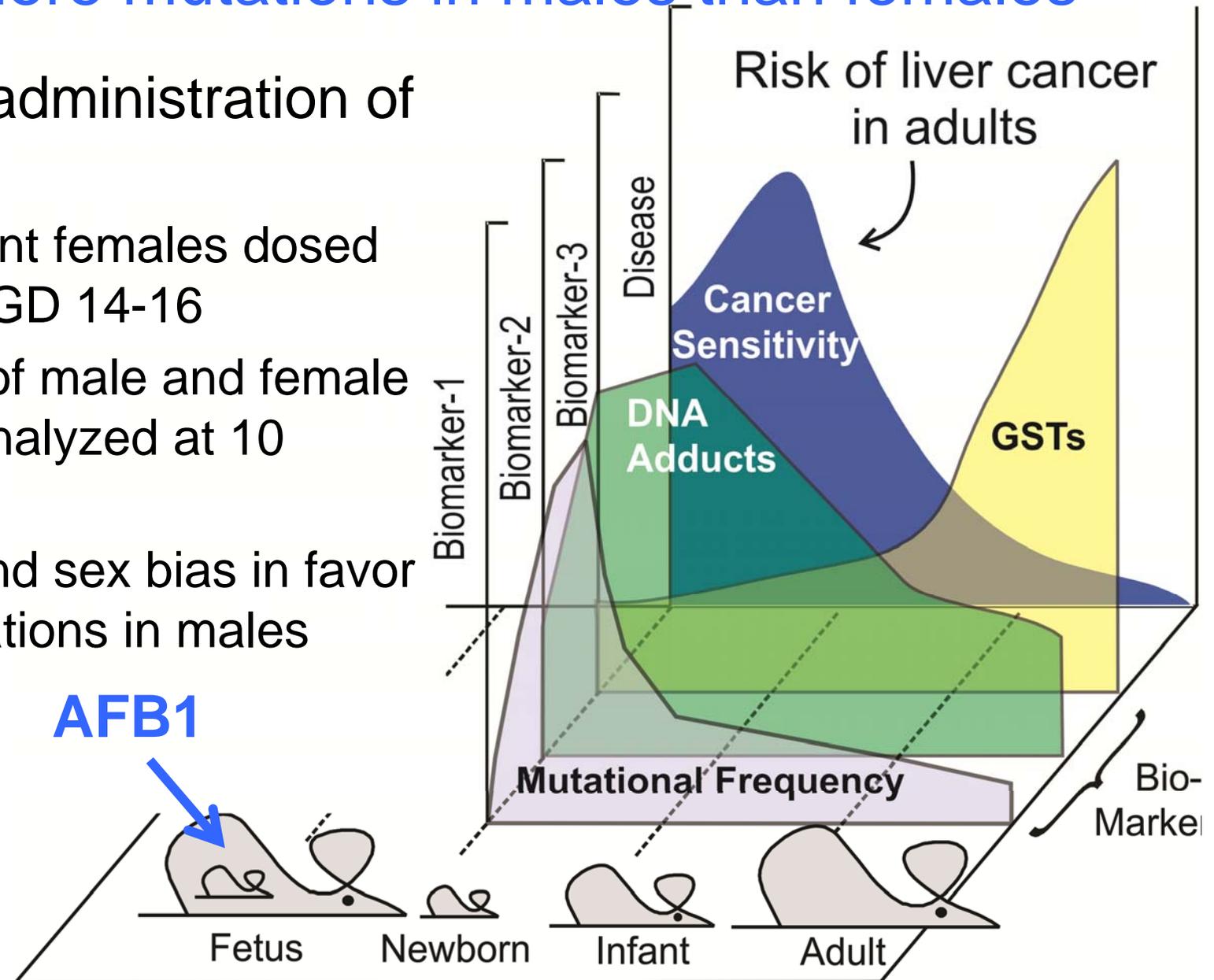
Maybe a “mutator phenotype” will develop





## There is one system where we have seen more mutations in males than females

- In utero administration of AFB1
  - Pregnant females dosed at day GD 14-16
  - Livers of male and female pups analyzed at 10 weeks
  - Profound sex bias in favor of mutations in males

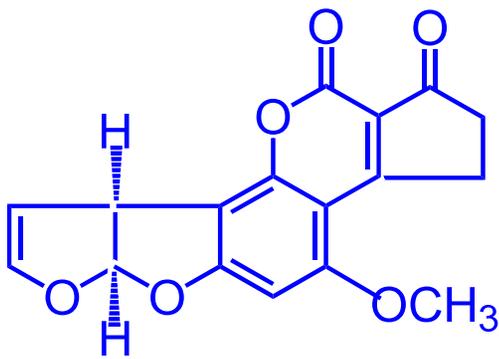


# Lessons Learned: B6C3F1 is a useful model

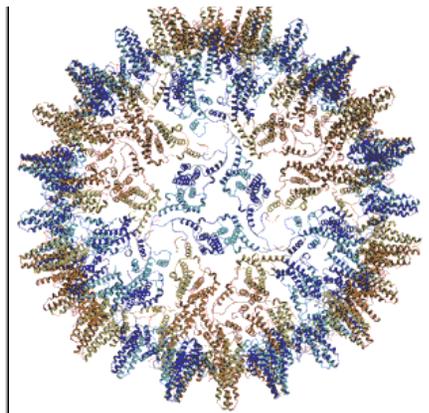
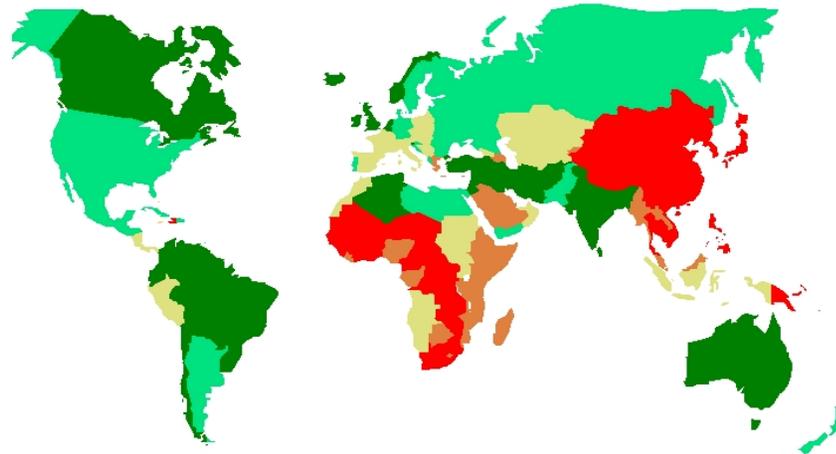
- Measurements of adducts correlate with gene expression changes and disease sensitivity
- Adducts correlate with mutations that (qualitatively) match those seen in human tumors
- Would endocrine disruptors affect AFB1 biology?
- Mouse can be adapted chemically (sulphorafane) or genetically (GstA2/3) to probe mechanism and predict chemosensitivity to disease
- The model is appropriate to study transplacental toxicology

# Where do we need to go? – More complex systems – with this mouse or other models

## *Incidence of Liver Cancer Overlaps with Both Aflatoxin Exposure and Hepatitis B Infection*



Aflatoxin B<sub>1</sub>



HBV

- The toxin and virus are synergistic
- If you are exposed to both, your relative risk of liver cancer goes up 60 to 100 fold
- There are no drugs that are truly effective against liver cancer
- The best way to address the disease is to avoid the agents that cause it ... or enhance your biochemical pathways of resistance

## Thanks To:

- Leslie Woo
- Jeannette Allen Fiala
- Apple Supawadee  
Chawanthayatham
- Anne Roongtiwa  
Wattanawaraporn
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- John Groopman
- Robert Croy
- Gerald Wogan