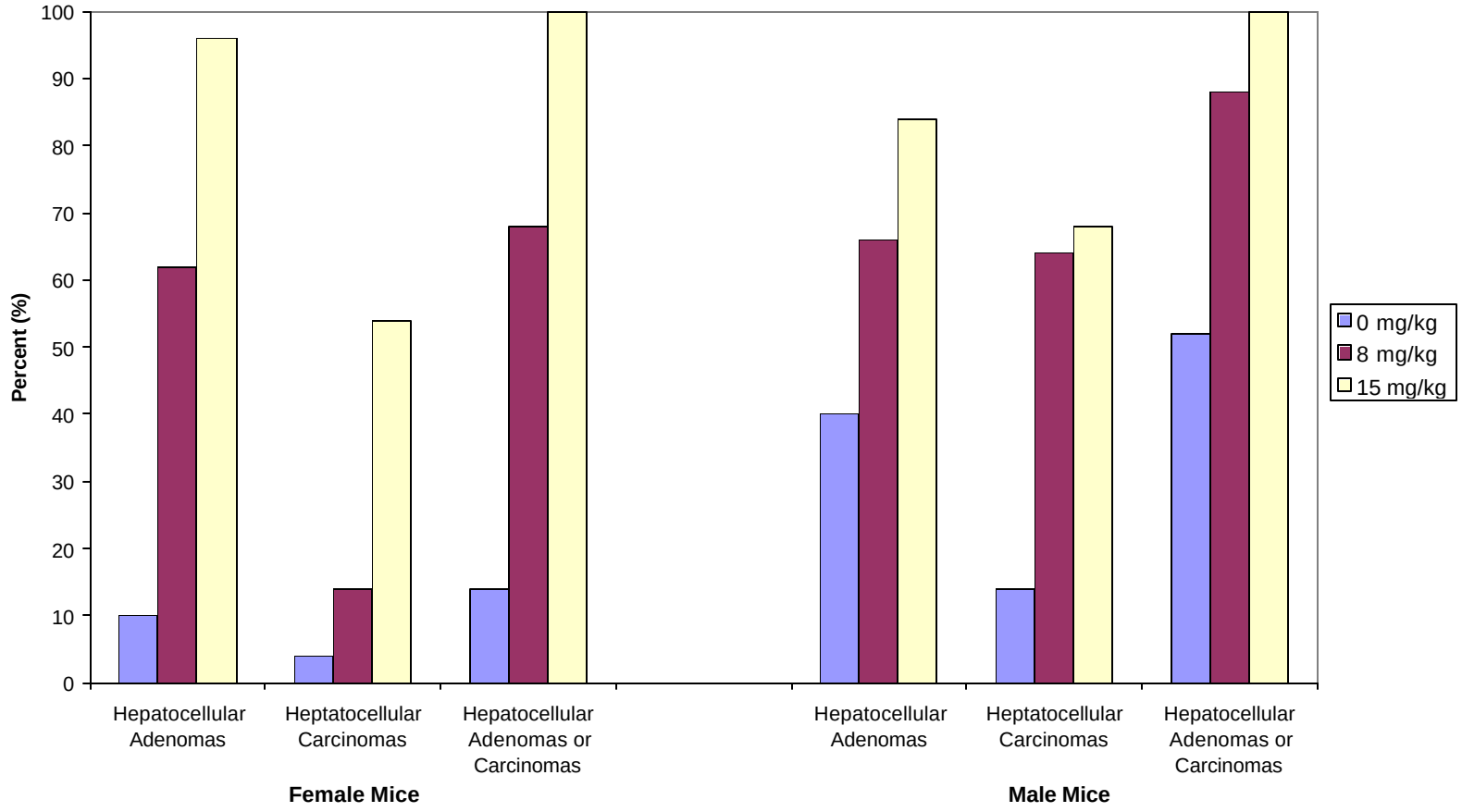
Furan-Induced Cytotoxicity, Cell Proliferation, and Tumorgenicity in Mouse Liver

Dr. Glenda Moser

2 Year NTP Bioassay

- Non-neoplastic
  - Rats
    - Kidney
    - Biliary Tract
    - Liver
  - Mice
    - Biliary Tract
    - Liver
- Neoplasms
  - Rats
    - Cholangiosarcoma – 2, 4, 8 mg/kg
    - Mononuclear cell leukemia
  - Mice
    - Benign pheochromocytoma
    - Hepatocellular – 8 mg/kg

Incidence of Liver Tumors in B6C3F1 Mice Exposed to Furan in the National Toxicology Program Bioassay

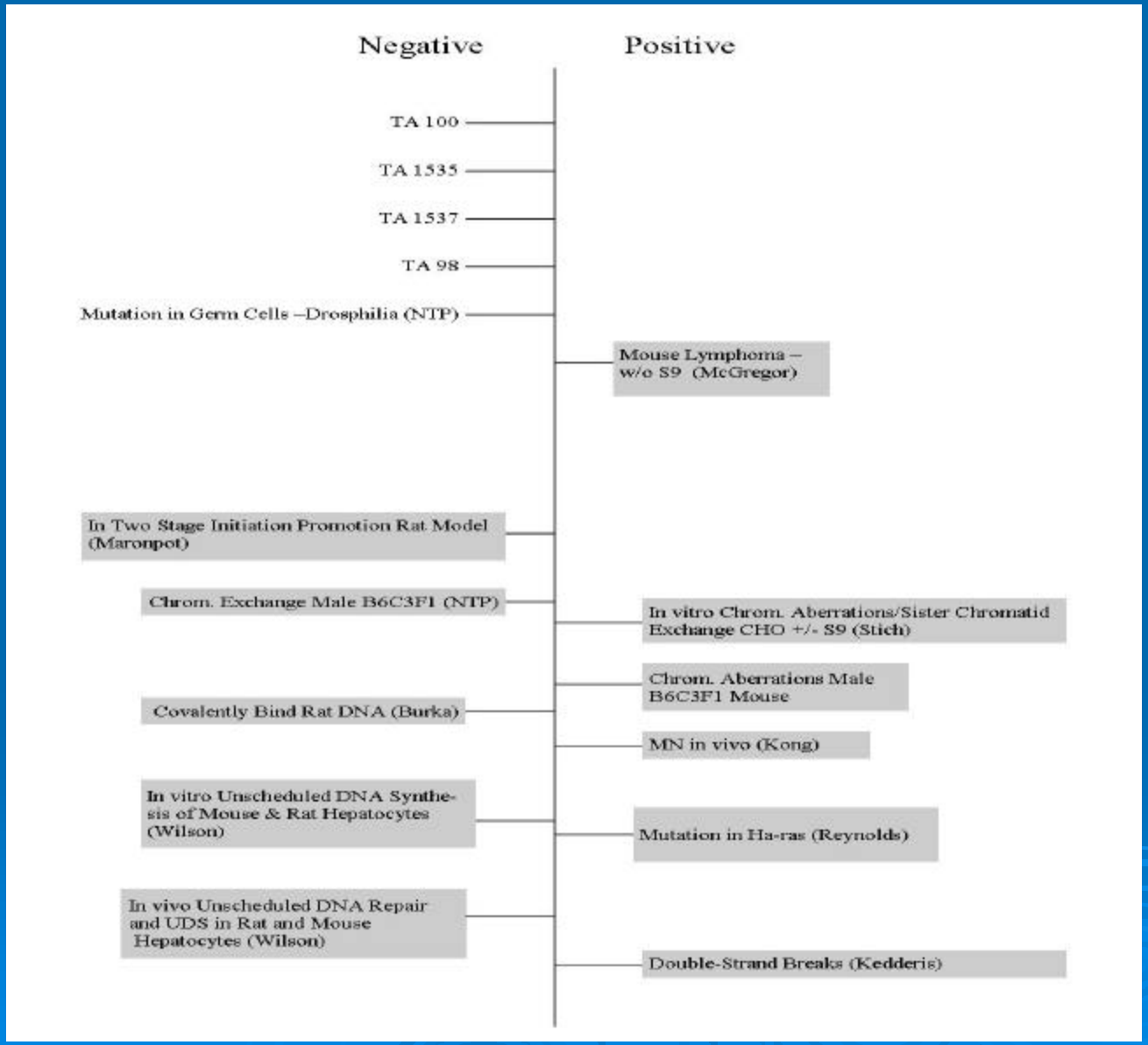


Cancer Multistage

Genotoxic

- Direct DNA damage
- Single exposure
- Metabolism
- **Mechanisms of Genotoxicity**
  - Mutations
  - Chromosomal translocations or loss
  - Gene amplification; fusion of viral promoters
  - DNA adducts
  - Promoter insertion

Assays for Genotoxicity after Furan Exposure



Cancer Multistage

- **Non-Genotoxic or Epigenetic**
  - Clonal expansion
  - Sustained exposure, high doses
  - Reversible
  - Specificity
    - B6C3F1 mouse
    - Liver

Cancer Multistage

Non-genotoxic Mechanisms

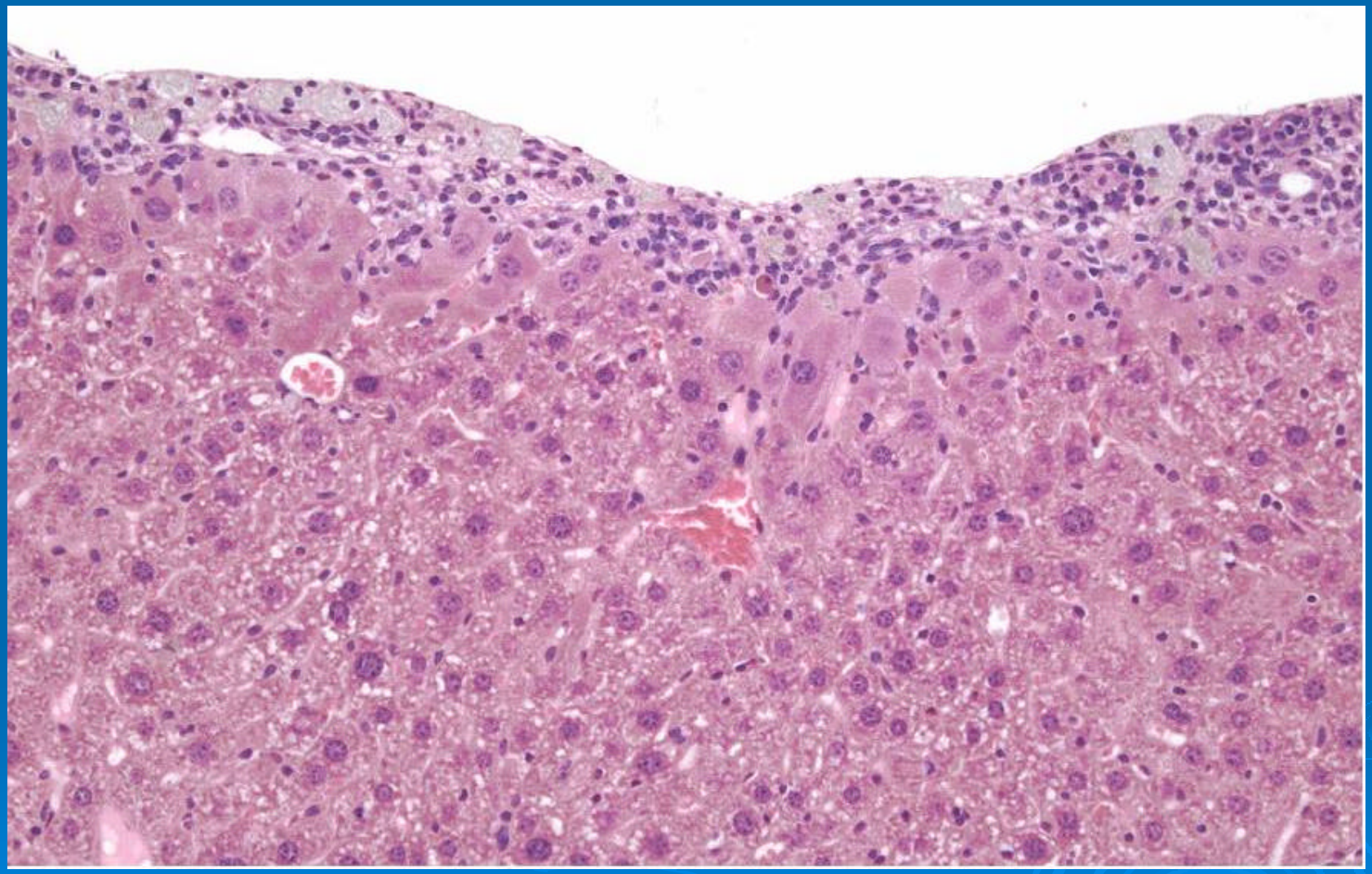
- Activated oncogenes, loss of suppressor genes-ras
- Methylations
- Immunosuppression
- Alteration of signal transduction and gene expression
  - Growth factors
  - Receptors
- Cell-cell communication
- Receptor mediated-receptor
- Oxidative stress
- Altered DNA repair
- Differentiation
- Apoptosis
- Cytotoxicity/Cell proliferation
  - Secondary mutations

Liver Cytotoxicity

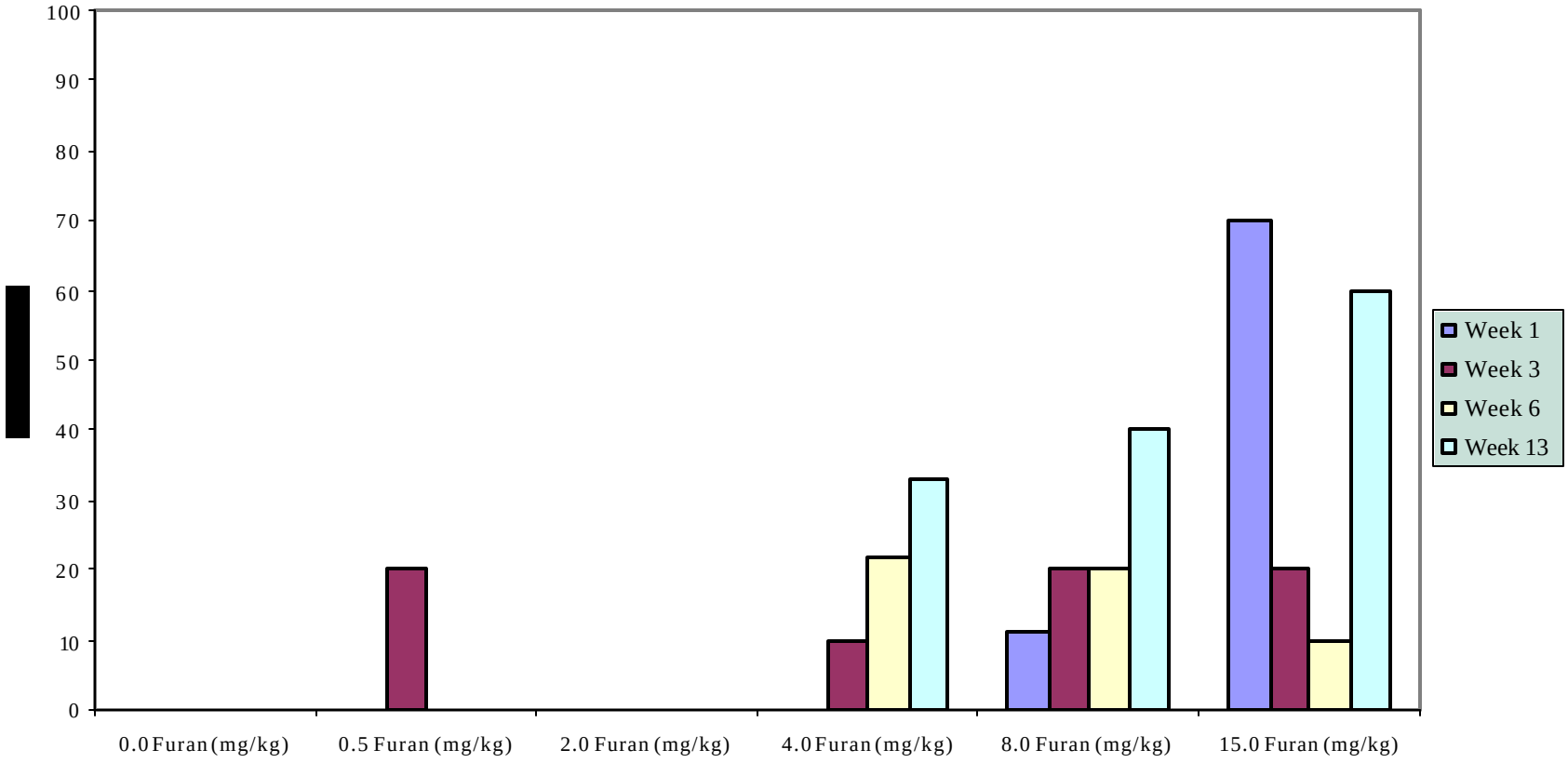
- **Clinical Chemistry**
  - ALT
- **Histopathologically**
  - Pynotic nuclei/ Condensed chromatin
  - Inflammatory response – left and caudate lobes
  - Degenerated hepatocytes/cytoplasmic vacuolation

Approach

- Male B6C3F1
- 10/Group
- 13 Weeks
- Gavage
  - 5X/wk
  - Dose levels 0, 0.5, 2, 4, 8, and 15 mg/kg
- BrdU
  - Osmotic pump
  - 7-day

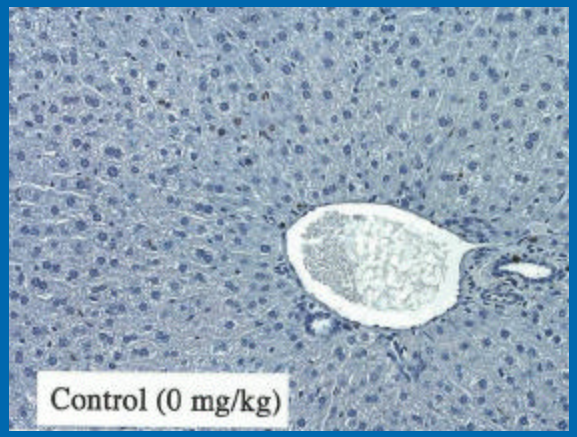


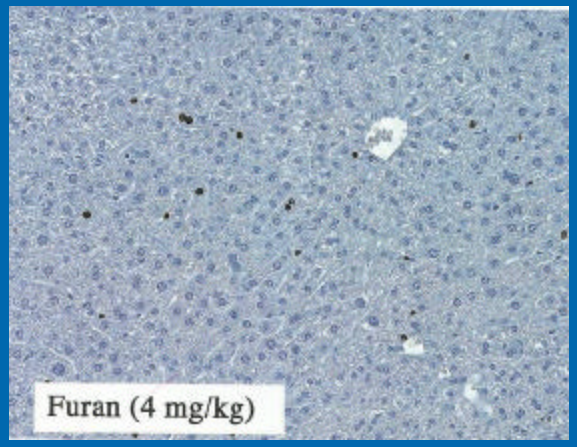
Incidence of Liver Cytotoxicity

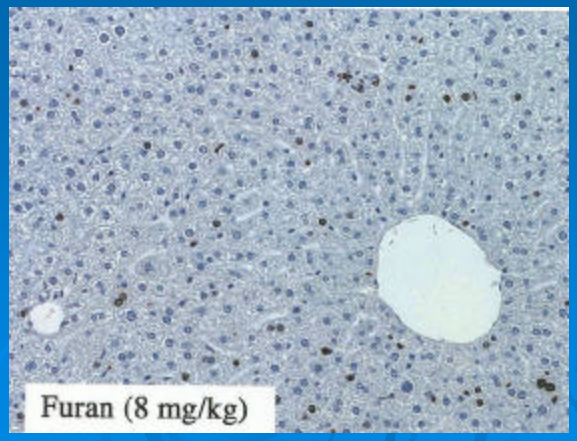


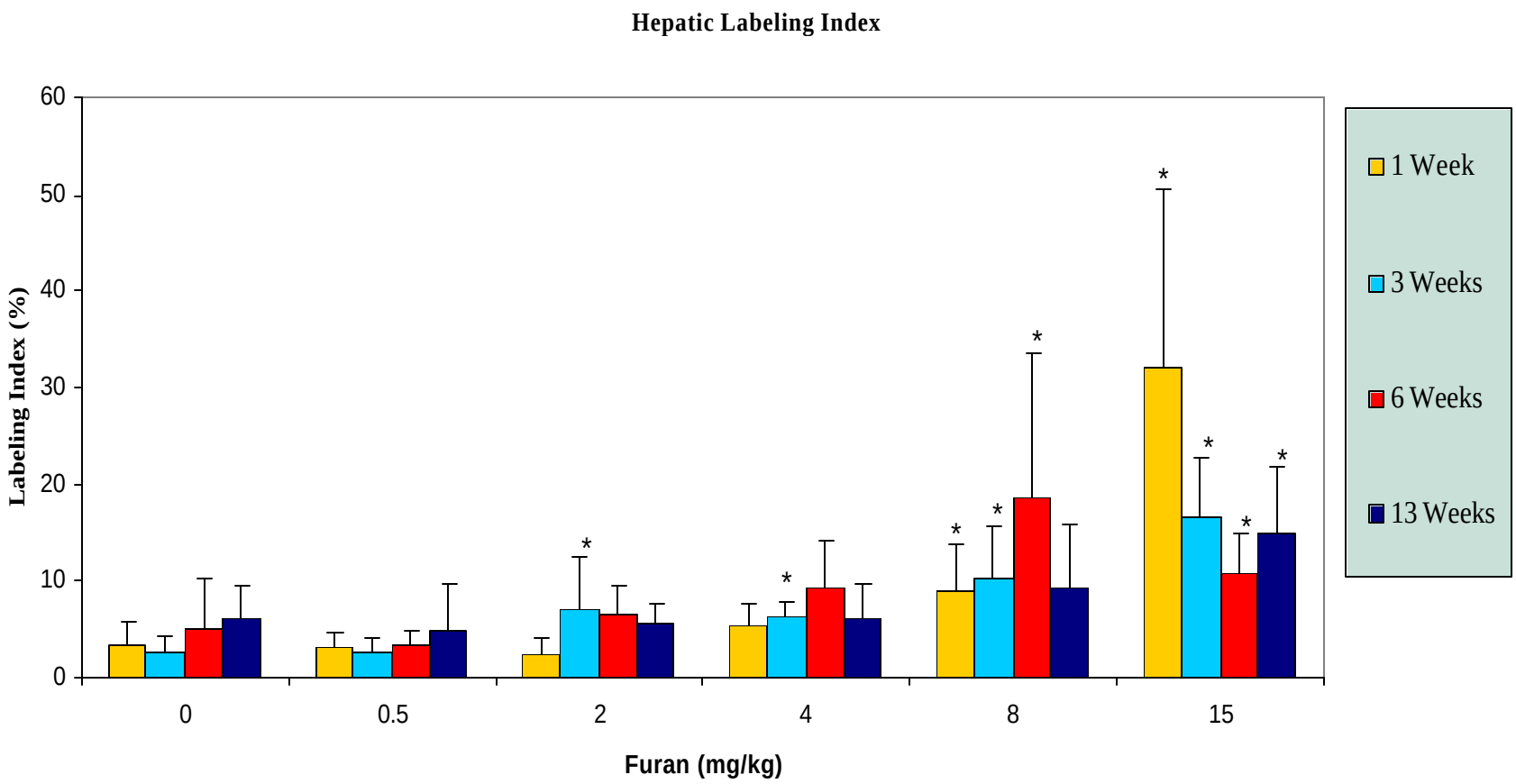
Cell proliferation

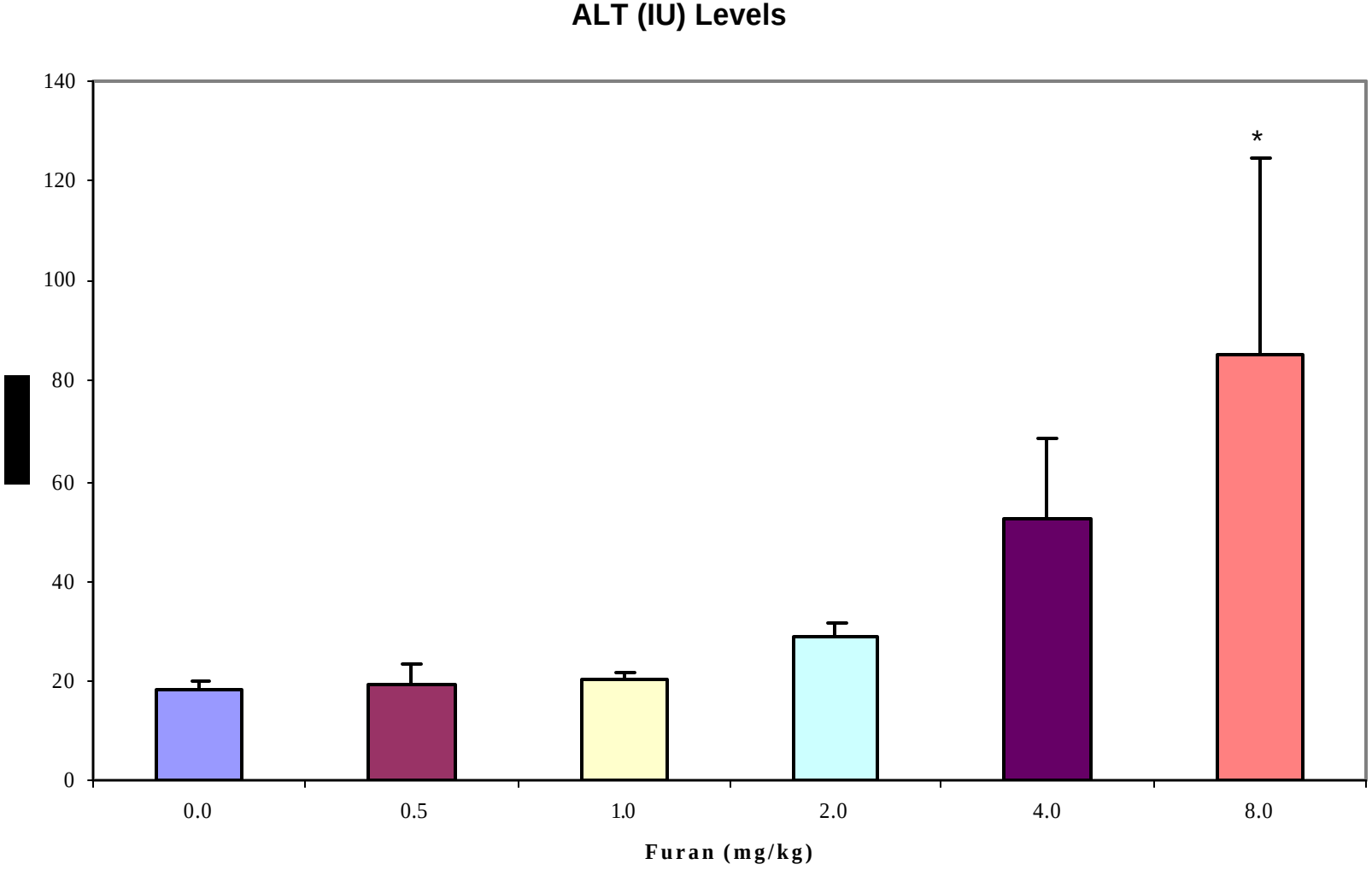
- **LI**
- **Methodologies**
  - BrdU thymidine analog *in vivo*
  - IHC for BrdU
  - Routes of administration
    - Pulse
    - Cumulative
- **Quantifying**


Control (0 mg/kg)

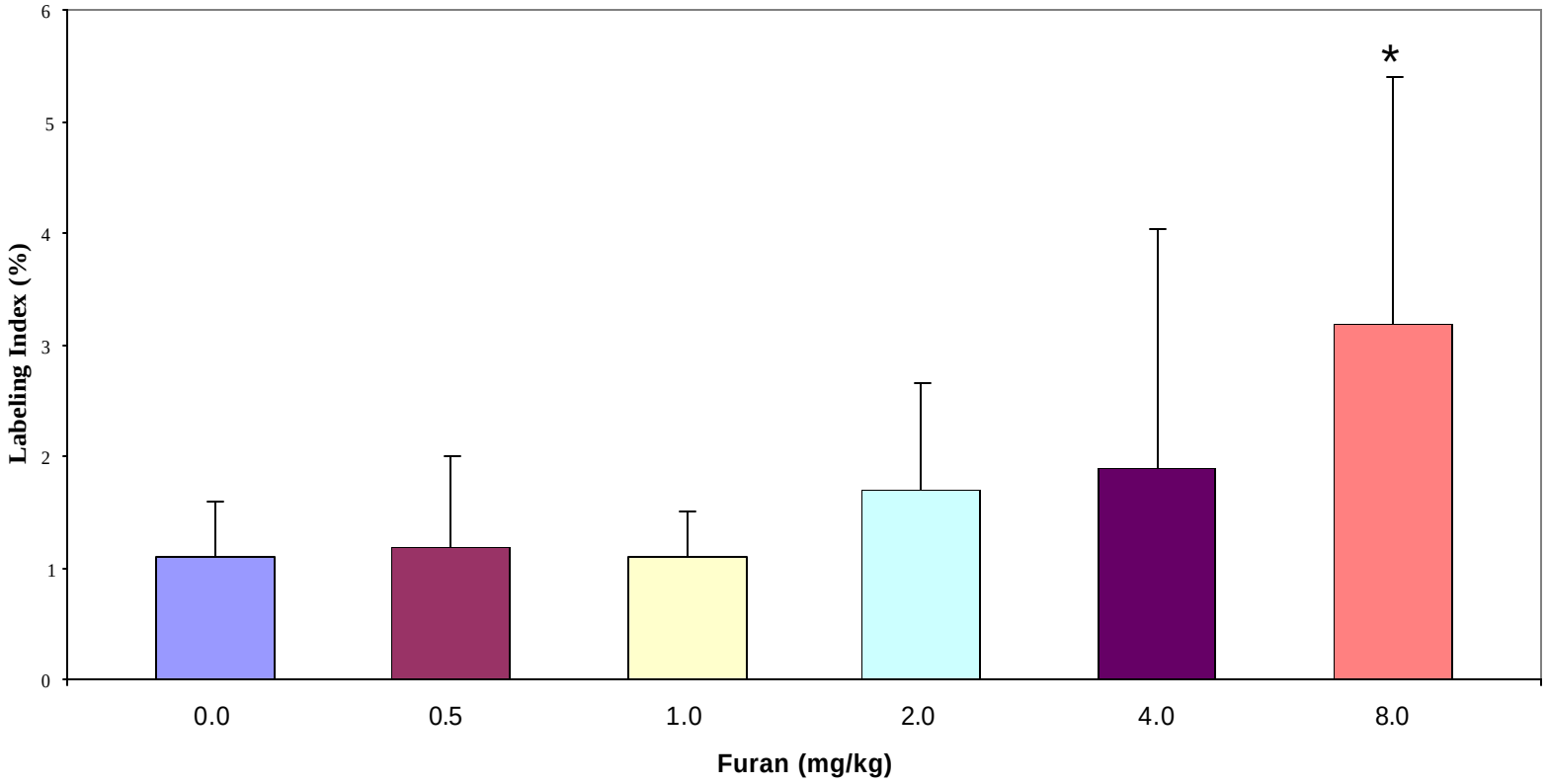

Furan (4 mg/kg)


Furan (8 mg/kg)


Hepatic Labeling Index
Labeling Index (%)
60
50
40
30
20
10
0
0
0.5
2
4
8
15
Furan (mg/kg)
1 Week
3 Weeks
6 Weeks
13 Weeks
*

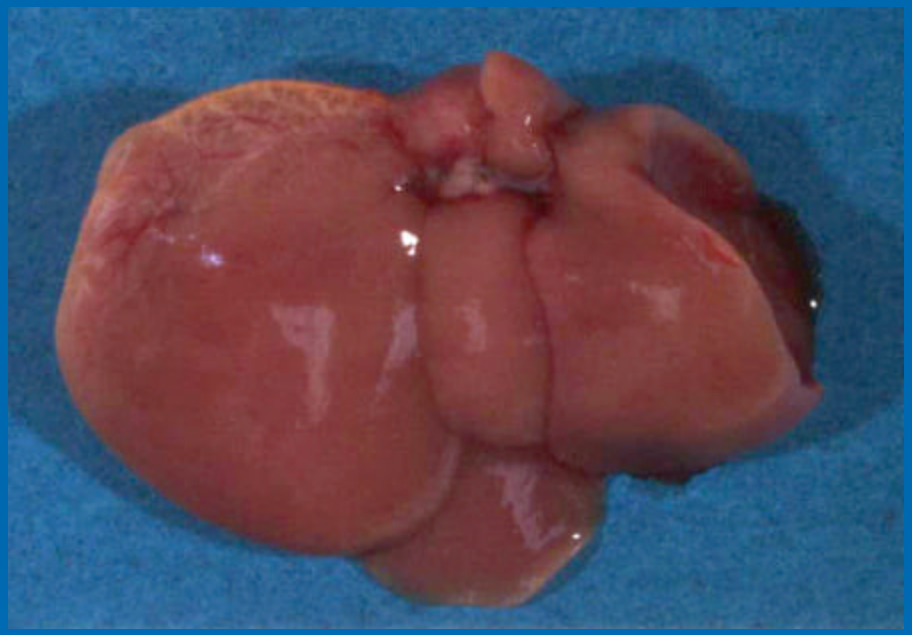

ALT (IU) Levels
140
120
100
80
60
40
20
0
*
0.0
0.5
1.0
2.0
4.0
8.0
Furan (mg/kg)

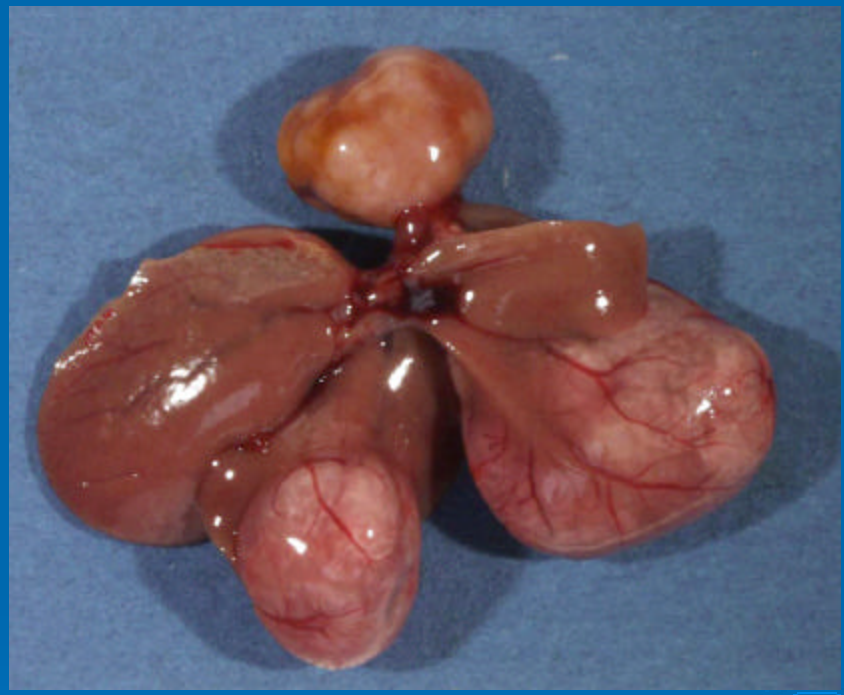
Hepatic Labeling Index in Female B6C3F1 Mice Exposed to Furan for Three Weeks

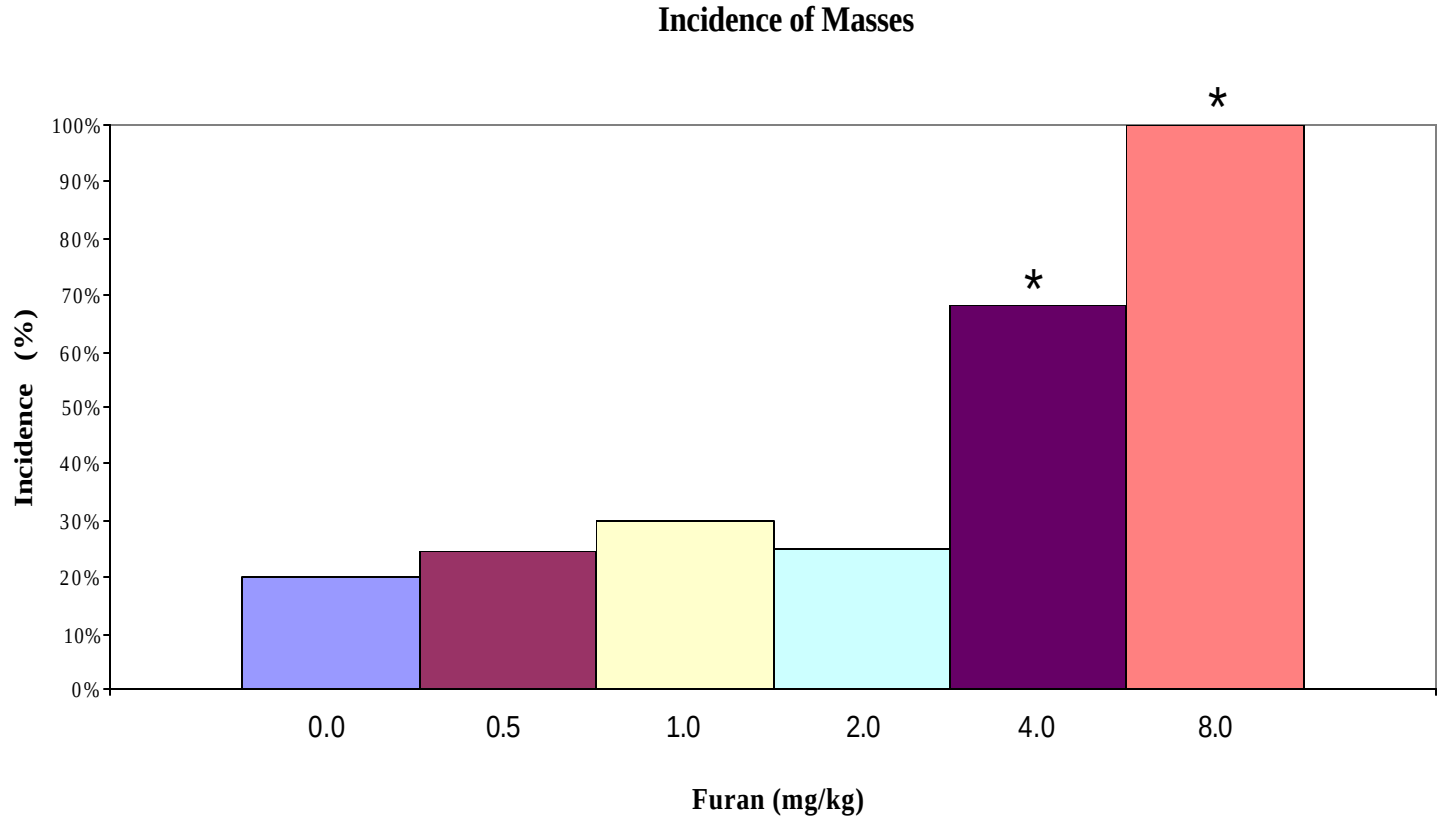


Carcinogenic Study

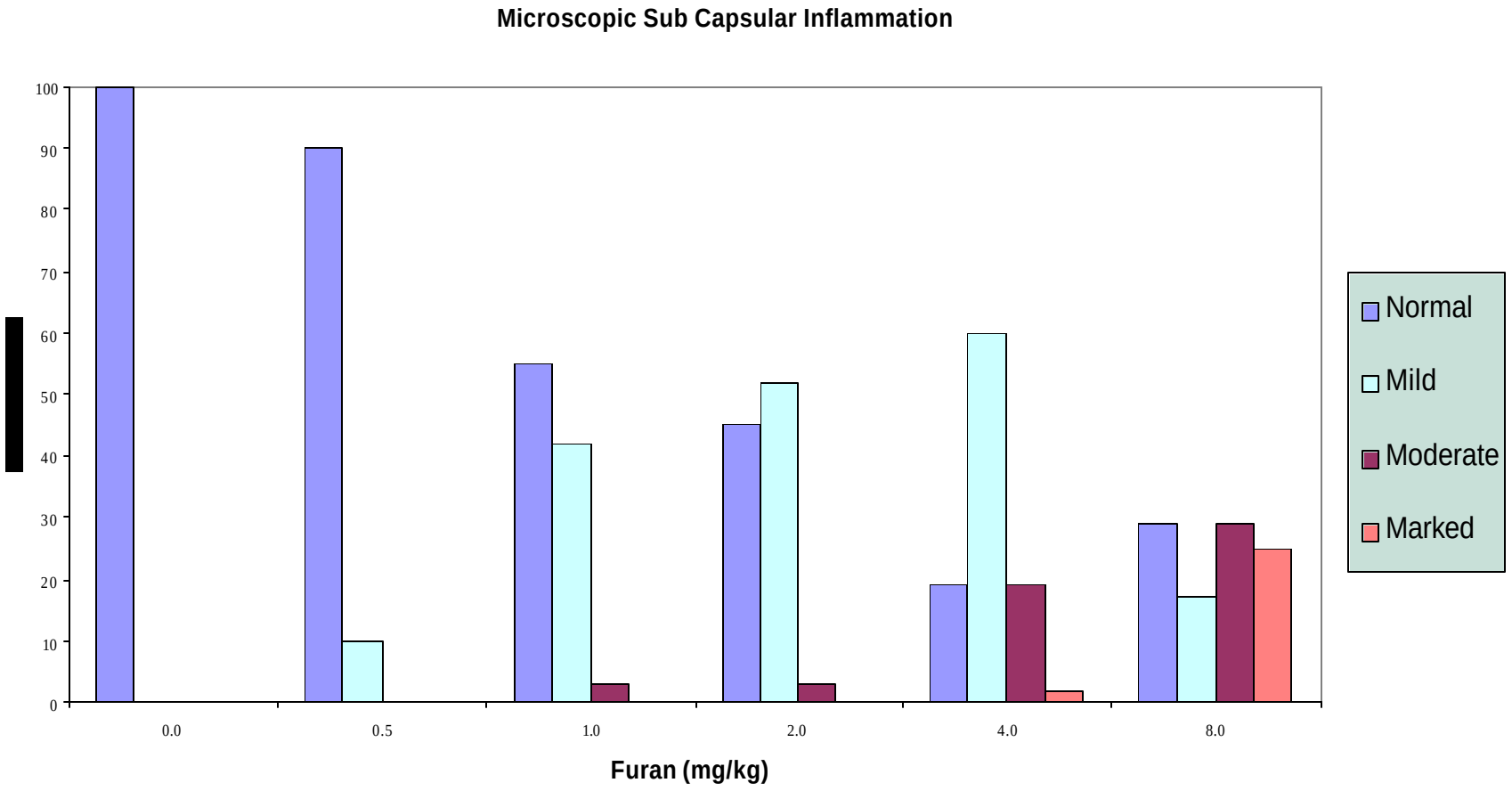
- Female B6C3F1
- Dose levels of 0.0, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg body weight
- 50- 100 per Group
- 2 years
- Gavage
  - 5X/wk

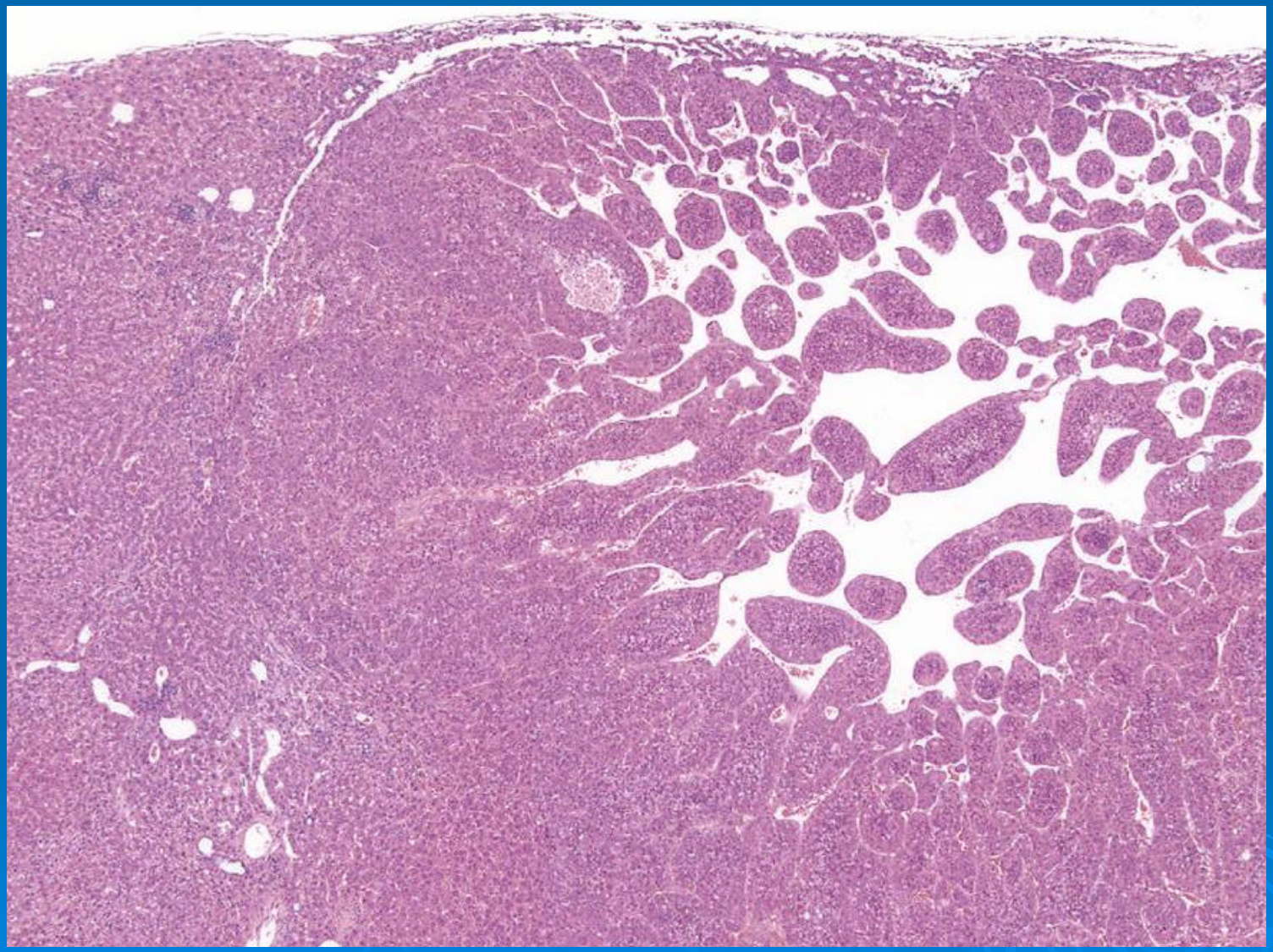




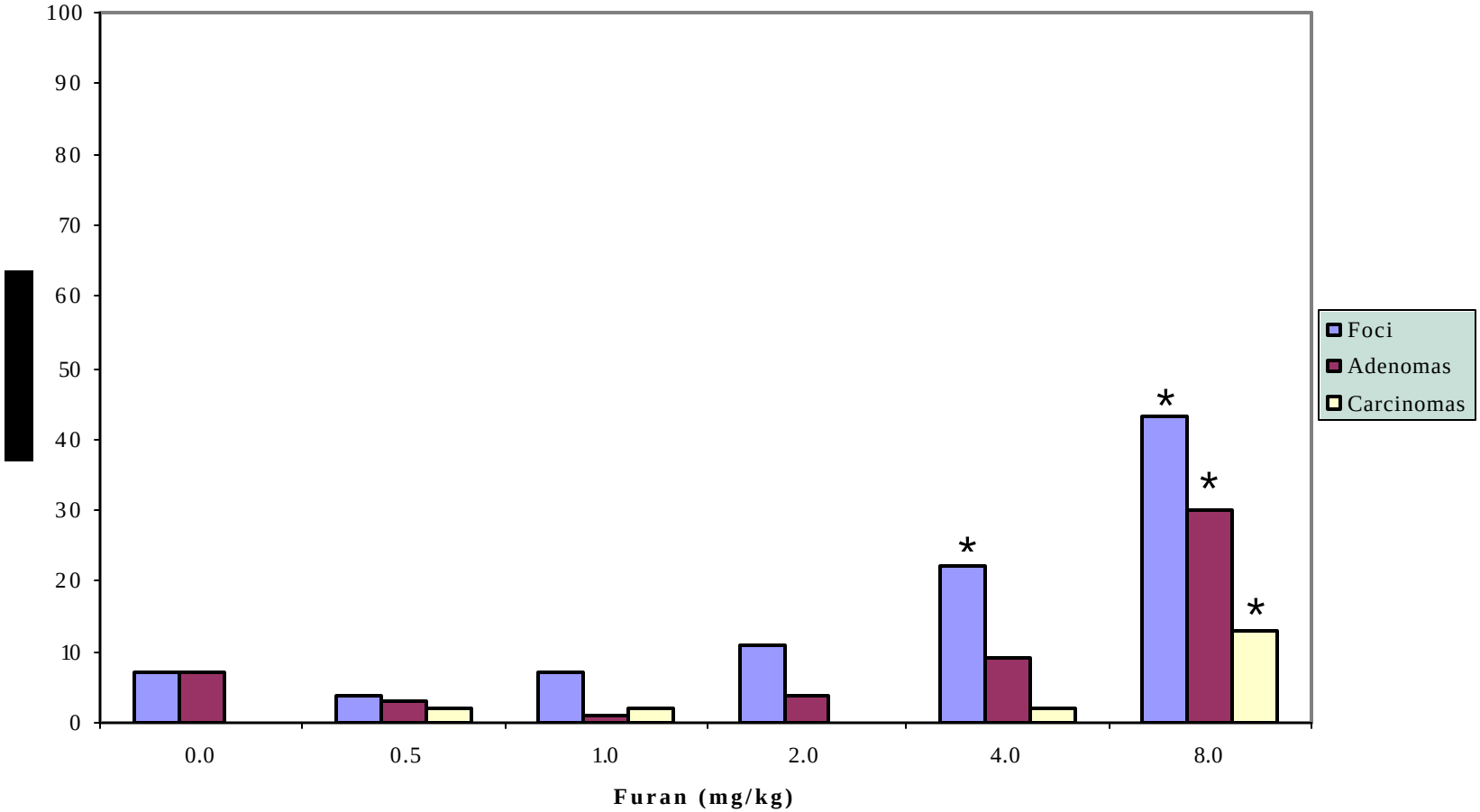

Incidence of Masses
Incidence (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
*
*
0.0
0.5
1.0
2.0
4.0
8.0
Furan (mg/kg)

Incidence of Cytotoxicity in Cancer Study





Incidence of Microscopic Hepatic Foci, Adenomas, and Carcinomas



Liver Findings in Male and Female Mice Exposed to to Furan

mg/kg	8.0	4.0	2.0	1.0	0.5
Cytotoxicity	? ?	+/- -	- -	- -	- -
ALT/SDH (IU)	?	- +/-	- -	- -	- -
LI (%)	? ?	- -	- -	- -	- -
Incidence of Liver lesions	?	+/-	-	-	-

Conclusion

- The study demonstrated a dose-dependent increase in furan-induced liver tumors in female B6C3F1 mice and a relationship between dose, cytotoxicity, and tumor induction for a model cytotoxic hepatocarcinogen.

Overview of Furan

- Reproduced results of NTP study
- Threshold for liver tumors
- At 13 weeks liver cytotoxicity and cell proliferation reversible
- Other chemicals with same proposed mechanisms
- Mutations and other events may be secondary to hepatocyte cytolethality or increased cell proliferation
- Biologically plausible model
- Furan-induced effects after short term exposure are inhibited by p450 Inhibitors
- Similar pharmokinetics in mouse and human in vitro; rat metabolizes furan slower

ILS

Areas of Interest

- Do p450 inhibitors decrease liver tumorigenesis in mouse liver?
- Are liver tumors due to bolus dose?
- Are the positive genotoxic results due to direct damage to DNA, high doses tested and/or secondary to cell proliferation?
- What are the molecular or gene expression changes in liver tumors? Do they occur in cholangiosarcomas?
- Is there a threshold for cholangiosarcomas or mononuclear leukemia? Is the mode of action similar to that of liver tumors?
- Do biliary tract epithelial cells have pharmokinetic parameters similar to that of hepatocytes?
- What is the relevance of mouse liver findings to cholangiosarcomas and leukemia or humans? PK
- Are there populations of humans that are susceptible to furan-induced effects? Is age a factor?

Acknowledgement

Special thanks to the

ILS toxicology techs animal care, laboratory assistants,

ILS histology department,

Dr. Robert Maronpot, NIEHS

Julie Foley, NIEHS

Dr. Tom Goldsworthy.

Questions?