THE ROLE OF EPIGENETICS IN TOXICOLOGICAL RISK ASSESSMENT OF FURAN IN FOOD

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OUTLINE

- Previous furan bioassays- remaining uncertainty
- NCTR/NTP furan studies
- Results - Metabolism, PK, histopathology, cancer, and epigenetics
- Neoplastic and non-neoplastic- critical effects
- FDA furan dietary intake assessment
- Role of epigenetic endpoints in risk assessment

NTP 2 y Carcinogenesis Studies TR 402 (1993)

- Male and female B6C3F_1 mice
- Doses: 0, 8, 15 mg/kg bw gavage 5 d/wk
- Hepatocellular adenoma or carcinoma:
  M 26/50; 44/50; 50/50
  F 7/50; 34/50; 50/50
- Adrenal gland benign pheochromocytoma
  M 1/49; 6/50; 10/50
  F 2/50; 1/50; 6/50
- Clear evidence of carcinogenicity

NTP 2 y Carcinogenesis Studies TR 402 (1993)

- Male and female F344 rats
- Doses: 0, 2, 4, 8 mg/kg bw gavage 5 d/wk
- Cholangiocarcinoma:
  M 0/50; 43/50; 48/50; 49/50
  F 0/50; 49/50; 50/50; 48/50
- Hepatocellular adenoma or carcinoma:
  M 1/50; 5/50; 22/50; 35/50
  F 0/50; 2/50; 4/50; 8/50
- Mononuclear cell leukemia:
  M 8/50; 11/50; 17/50; 25/50
  F 8/50; 9/50; 17/50; 21/50
- Clear evidence of carcinogenicity

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- Furan is hepatotoxic and hepatocarcinogenic based on effects in rats and mice
- BMDL10 for mouse liver tumors 0.96 mg/kg bw/d (7d/wk)
- Human exposure 1 and 2 µg/kg bw/d for mean and high consumers, including children
- MOEs 960-480
- “MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite”

Evidence for Genotoxicity in Furan Hepatocarcinogenesis

- 3 DNA adducts formed *in vitro* from *cis*-2-butene-1,4-dial
- *cis*-2-butene-1,4-dial is direct acting mutagen in *Salmonella*
- Furan induced H-*ras* mutations in B6C3F$_1$ liver tumors
  (C → A transversions, A → G transitions)
- Neonatal mouse carcinogenesis (6 x 200 mg/kg bw/d ip on PND 3, 6, 9, 12, 15, 18; 1 x 400 mg/kg ip on PND 12)

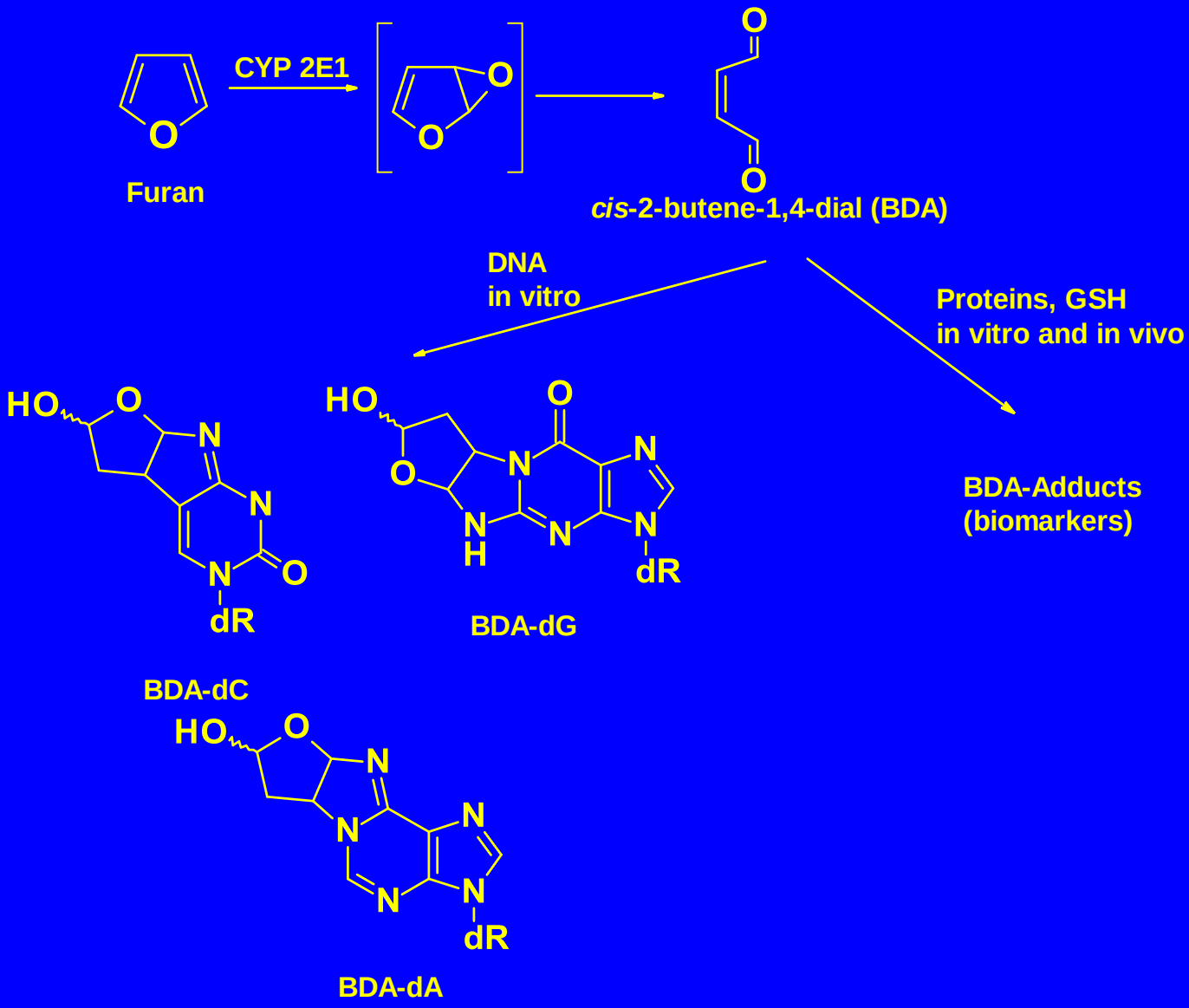
Evidence for Furan Hepatocarcinogenesis Secondary to Regenerative Hyperplasia

- Rat bioassay doses produced:
  Biliary tract – chronic inflammation, fibrosis, hyperplasia and metaplasia
  Hepatocyte – cytomegaly, vacuolization, degeneration, hyperplasia, and necrosis
- Mouse bioassay doses produced:
  Biliary tract – chronic inflammation, cysts, fibrosis, hyperplasia, and metaplasia
  Hepatocyte – cytomegaly, vacuolization, degeneration, hyperplasia, and necrosis

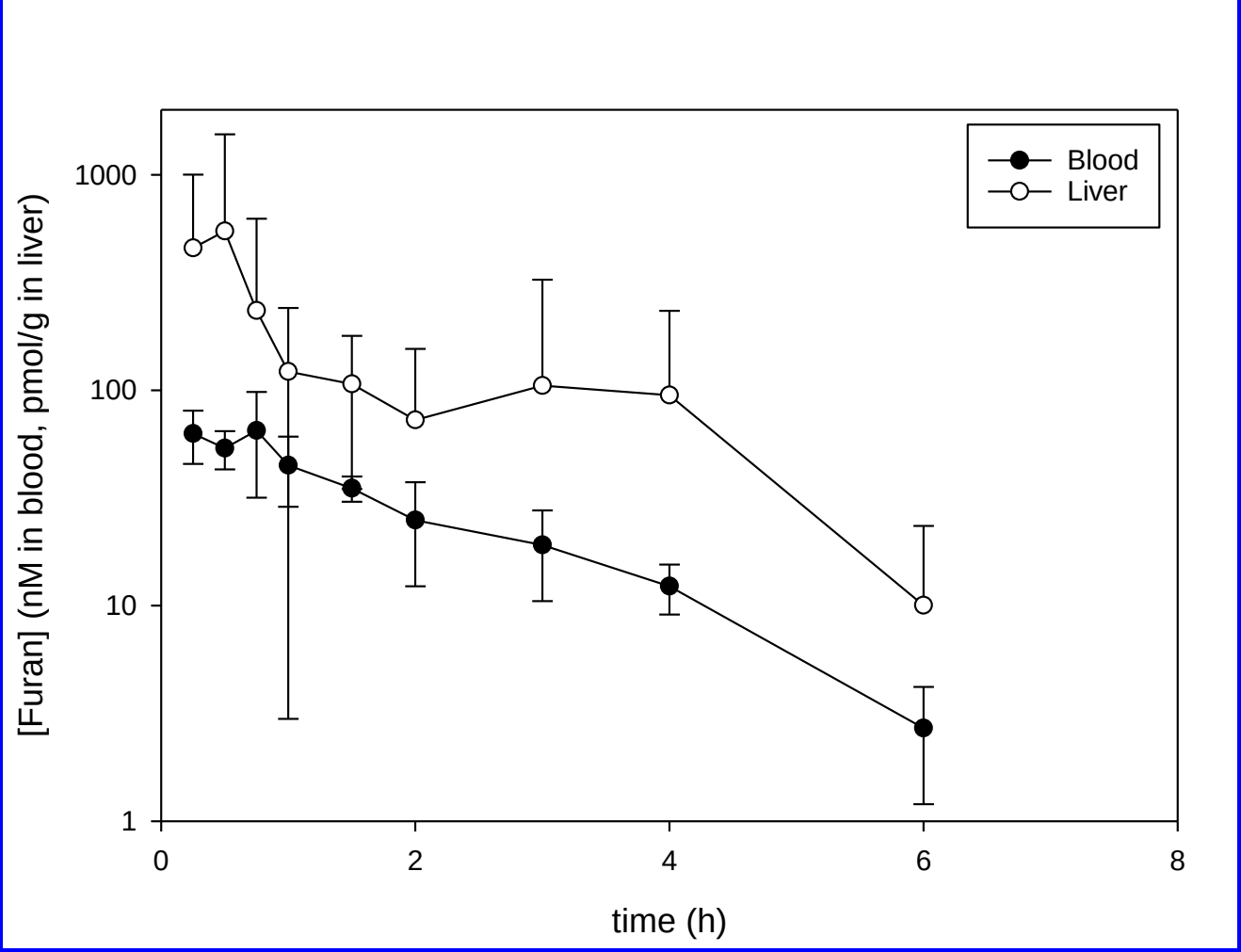
NCTR/NTP Toxicological Testing of Furan

- Chronic bioassay (0.02-2 mg/kg bw/d for 2 y)
- Toxicokinetic study in male F344 rats
- Determine furan-DNA adduct formation in liver
- Big Blue rat mutagenesis (doses 2-30 mg/kg bw for 60 d)
- Time x Dose effect on liver histopathology (0, 0.092, 0.44, 0.92, 2, 4.4 mg/kg bw/d gavage for 45-360 d mo)
- Determine reversibility of hepatotoxicity biomarkers in stop-dosing arm (8 mg/kg bw/d for 90 d) then 0, 45, 90, 180, 360 d recovery
- Epigenetic changes – parallel evaluation

Metabolic Activation of Furan



Pharmacokinetics of Furan in Rat Serum and Liver



Results: TK and Genotoxicity

- Adult rat TK – t1/2 elimination of furan from liver (0.6 h) and blood (1.3 h)
- Liver/serum AUC ratio 5.6
- Furan-dC, the major DNA adduct, is undetectable after single and repeated dosing (≤4.4 mg/kg @ 24 h and 45-360 d)
- Micronuclei (bone marrow): negative
- Bone marrow comet assay: negative
- Liver comet assay: positive at 4-16 mg/kg
- *Hprt* (WBC)*:* negative
- Pig-a (erythrocyte, WBC): negative
- *cII* (liver)*:* negative

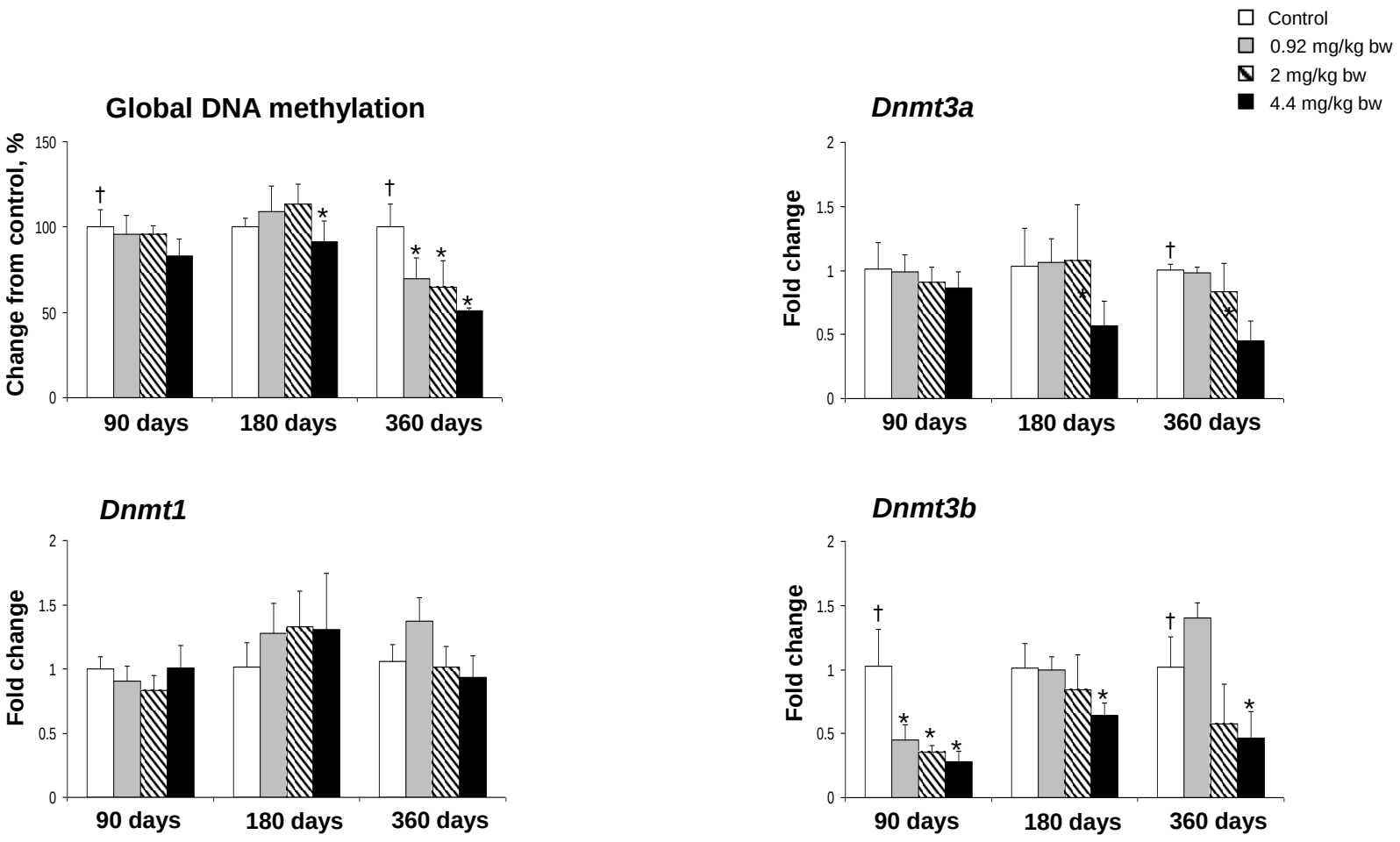
Results - Liver Histopathology

- Dose- and time-dependent increases in incidence and/or severity: centrilobular fatty degeneration (45-90 d); fibrosis (45-90 d); cholangiofibrosis (180-360 d); Ki-67-positive hepatocytes (45-360 d)
- LOEL for fibrosis 0.092 mg/kg @ 360 d
- LOEL for cholangiofibrosis 0.44 mg/kg @ 360 d
- Lobe differences
- Stop Dose Arm (8 mg/kg bw x 90 d): fatty degeneration, Ki-67-labeling, fibrosis decreases; cholangiofibrosis, initially absent, observed after 90 d - increased incidence/severity at 180-360 d
- 2-y study (2 mg/kg bw/d) - cholangiofibrotic and surrounding nomal tissue ; control tissue

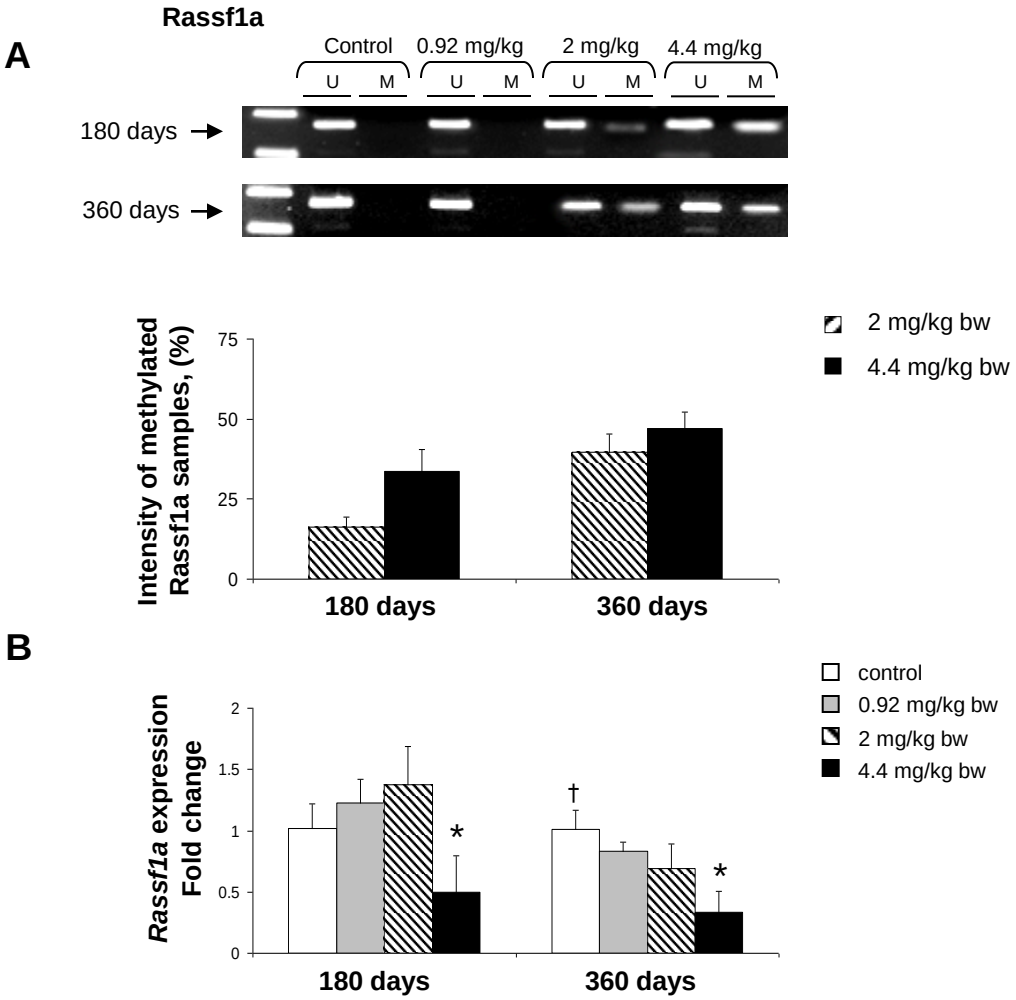
Implications for Epigenetic Effects

- Absence of apparent genotoxicity in liver
- Metabolism and TK indicate liver as the focus
- After continuous dosing, histopathological changes in liver depend on dose x time
- After transient dosing some histopathological changes diminish, some persist, and some do not appear until later
- Caveat – doses often far above dietary levels

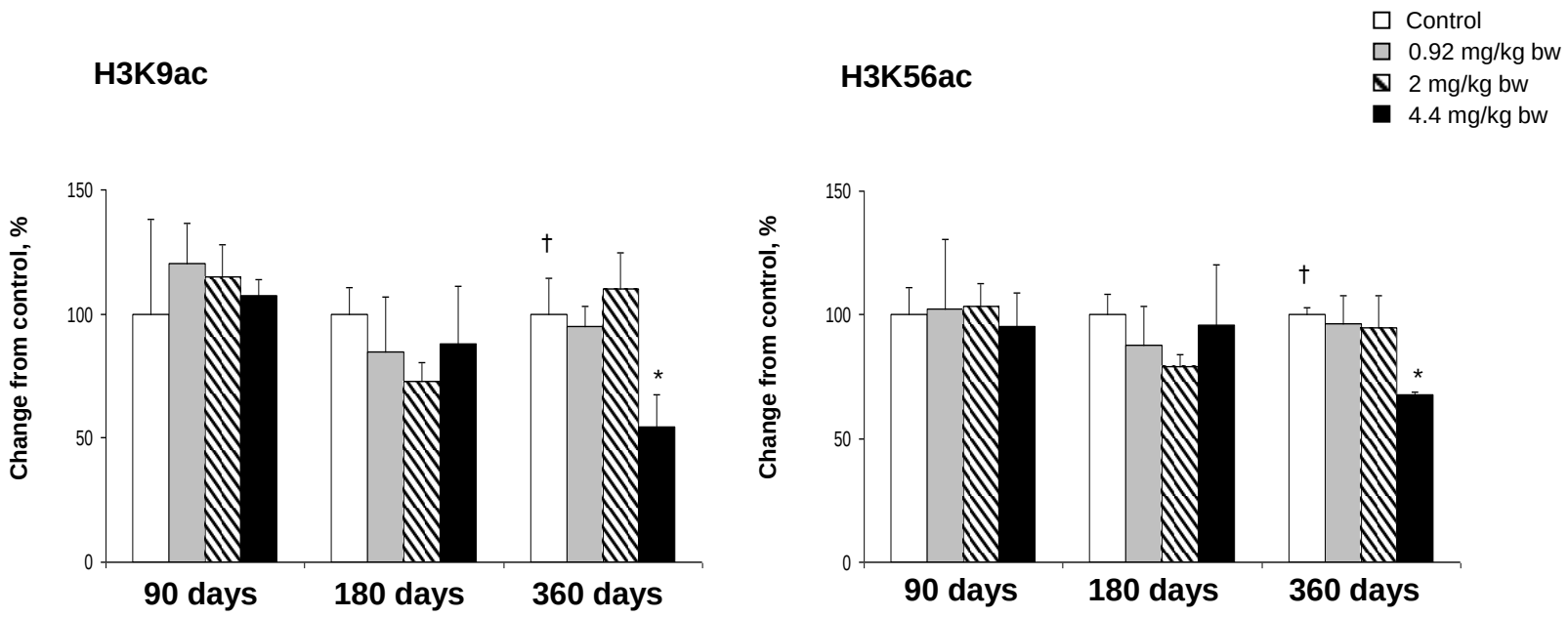
DNA Methylation and DNA Methyltransferase Expression in Furan-treated Rat Liver



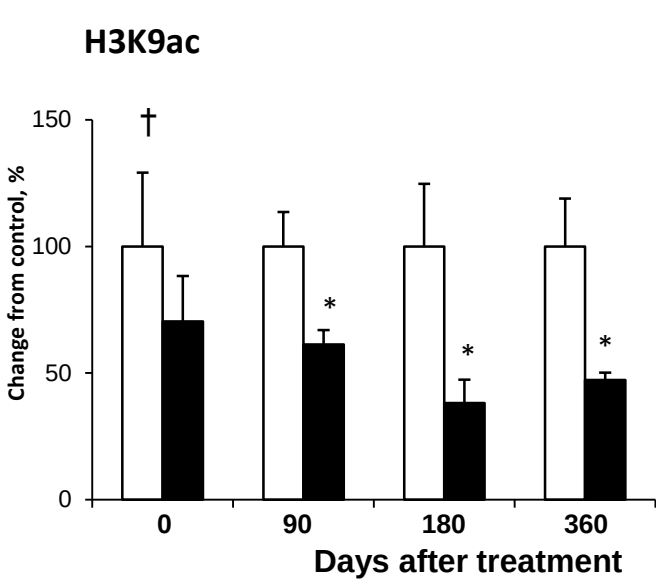
Correlation Between Gene-specific DNA Methylation and Expression in Furan-treated Rat Liver

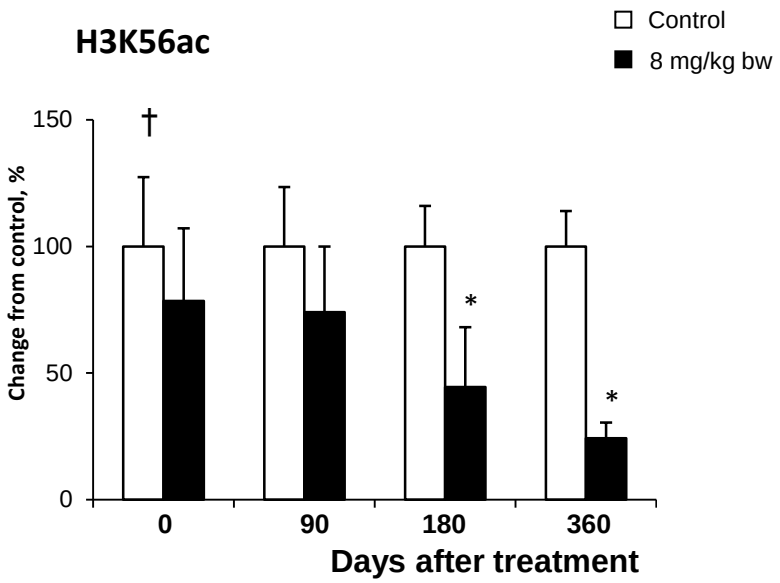


Histone Lysine Acetylation Changes in Furan-treated Rat Liver (Dose-Response Study)

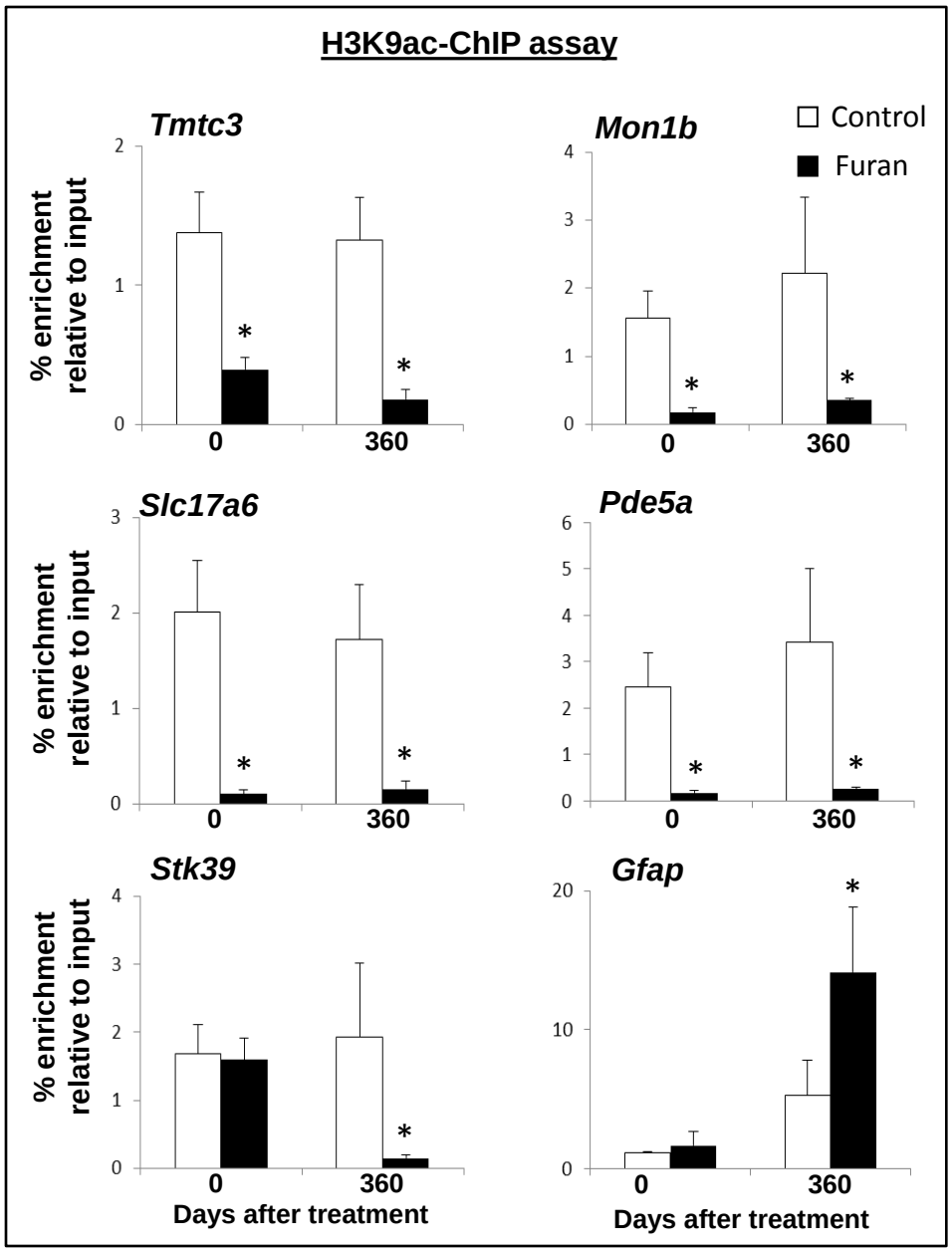


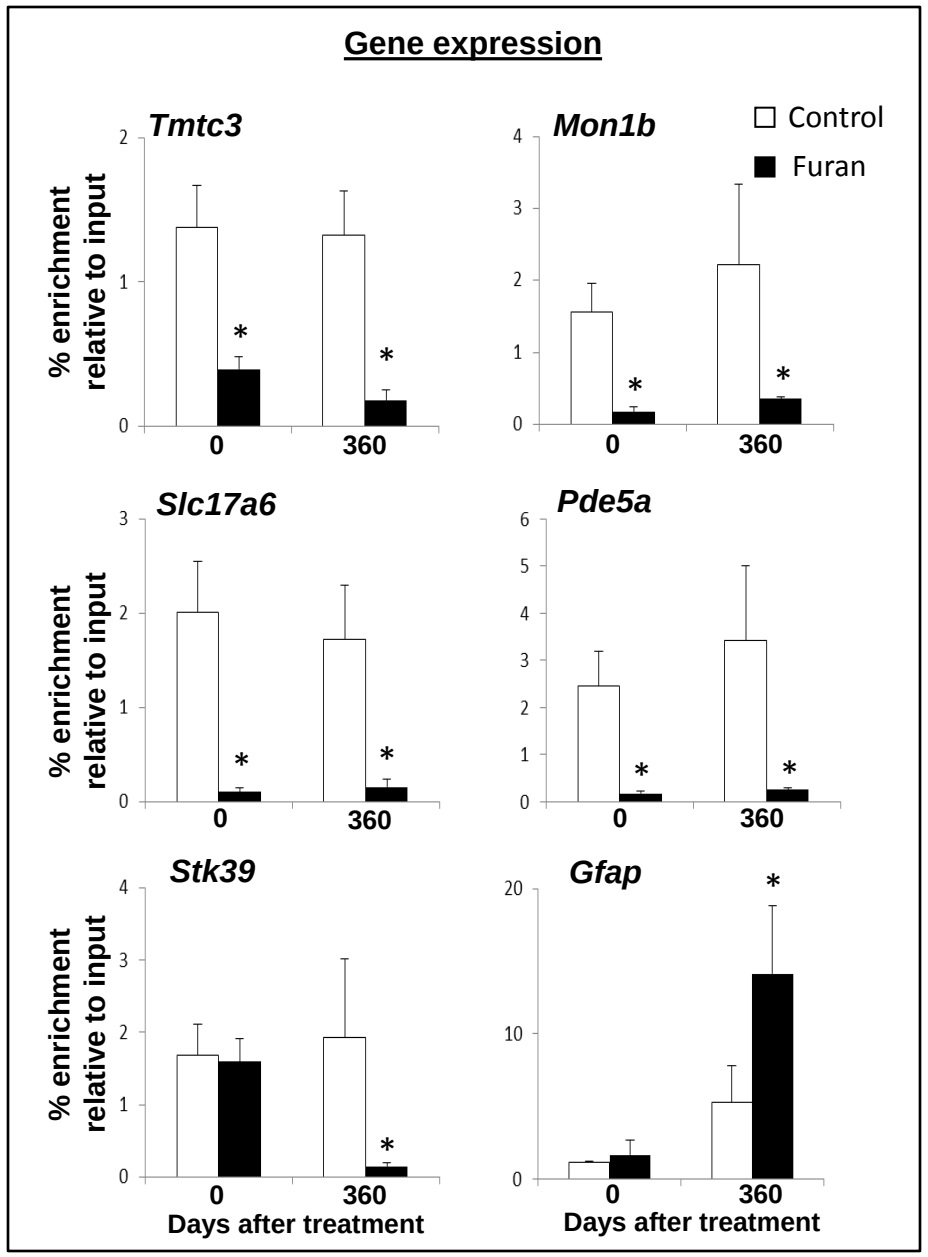
Histone Lysine Acetylation Changes in Furan-treated Rat Liver (Stop-Dose Study)





Persistent Gene-specific H3K9 Acetylation and Expression in Furan-treated Rat Liver (Stop-Dose Study)

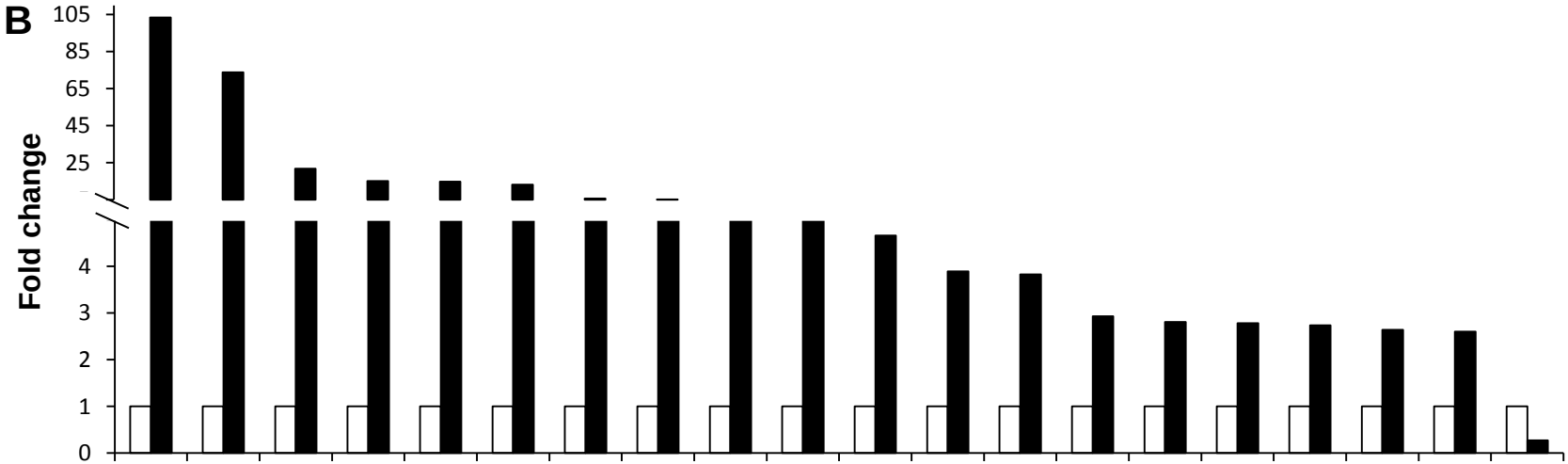




Expression, Gene-specific Methylation of Differentially Expressed CpG Island-containing Genes in Furan-induced Cholangofibrotic Tissue

A

B



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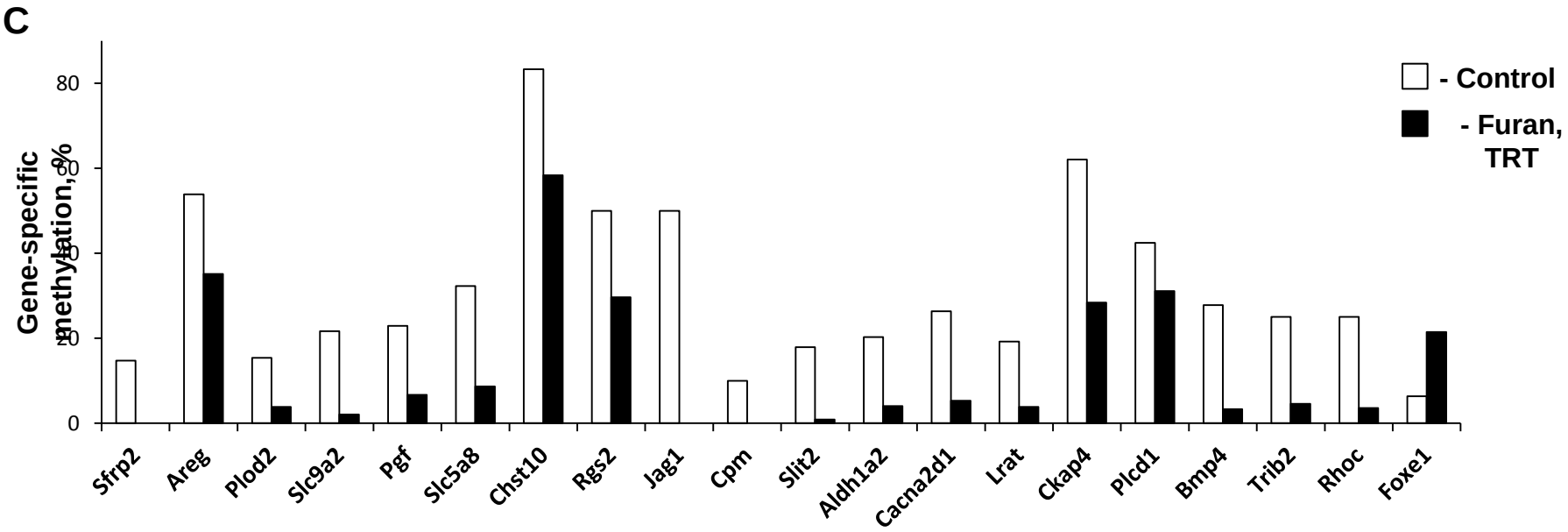

Results – Epigenetic Changes in Furan–treated Rat Liver

- Dose-dependent decreased DNA demethylation, decreased histone lysine methylation/acetylation
- Hypermethylation-dependent down-regulation of tumor supressor gene (*Rassf1a)*
- Decreased expression of histone methyl/acetyl-transferases
- Persistent (1 y after stop-dose) decrease in histone acetylation associated with altered gene expression/pathway-specific changes for fibrosis and carcinogenesis (e.g., stellate cell activation, *Gfap*, *YAP*)
- Pairing microarray transcriptomics and bisulfite sequencing of DNA methylation identify key pathways associated with furan hepatotoxicity and carcinogenicity
- Dose- and temporal-responses

Incidences of Furan-induced Cholangiofibrosis in F344 Rats

FURAN (mg/kg bw/d; 5d/wk for 2 y)

Lesion	0	0.02	0.044	0.092	0.2	0.44	0.92	2
Cholangiofibrosis	0/149 (0%)	0/150 (0%)	0/99 (0%)	1/100 (1%) p=0.42	38/50 (76%) p<0.001	49/49 (100%) p<0.001	47/50 (94%) p<0.001	49/49 (100%) p<0.001
Average severity				1.0	1.7	2.6	6.4	6.9

Incidences of Furan-induced Malignant Mesothelioma (Peri-testicular) in F344 Rats

FURAN (mg/kg bw/d; 5d/wk for 2 y)

Tumour	0	0.02	0.044	0.092	0.2	0.44	0.92	2
Epididymis or Testes Malignant Mesothelioma	6/150 (4%)	8/150 (5%) p=0.37	1/98 (1%) p=0.16	2/100 (2%) p=0.30	0/50 (0%) p=0.16	2/50 (4%) p=0.65	2/50 (4%) p=0.62	6/50 (12%) p=0.033*

Furan Consumption from Adult Foods

Brewed coffee	0.15 µg/kg-bw/day
Chili	0.04 µg/kg-bw/day
Cereals[1]	0.01 µg/kg-bw/day
Salty Snacks	0.01 µg/kg-bw/day
Soups containing meat	0.01 µg/kg-bw/day
Pork and beans	0.004 µg/kg-bw/day
Canned pasta	0.004 µg/kg-bw/day
Canned string beans	0.004 µg/kg-bw/day
Pasta sauces	0.001 µg/kg-bw/day
Juices	0.001 µg/kg-bw/day
Canned tuna (water packed)	0.00008 µg/kg-bw/day

Aggregate Daily Furan Intake

- Based on concentration data posted through Spring 2007
- Adult Foods (2+ year olds)
  - Mean 0.26 μg/kg bw/day
  - 90th percentile 0.61 μg/kg bw/day
- Infant foods (0-1 year olds)
  - Mean 0.41 μg/kg bw/day
  - 90th percentile 0.99 μg/kg bw/day

Elements of a Furan Risk Assessment

- Cholangiocarcinoma absent (≤2 mg/kg bw/d)
- NTP 1993 – hepatocarcinogenic (≤4 mg/kg bw/d)
- Critical effect (neoplastic): malignant mesothelioma
  BMDL10: 1 mg/kg bw/d
  MOE 3850-1000 (genotoxic vs. non-genotoxic?)
- Critical effect (non-neoplastic): cholangiofibrosis
  BMDL10: 0.1 mg/kg bw/d
  MOE 380-100
- Critical effect (epigenetic) ? ~2 mg/kg bw
  mechanistic utility (specific gene and pathway analysis)
- Separation of treatment-specific effects from generalized cell damage is key (dose-response; early vs. late events; reversible vs. irreversible effects)
- Effects on liver progenitor (stem) cells