NTP
National Toxicology Program

NTP Research Concept: Furan

Daniel R. Doerge, Ph.D.

National Center for Toxicological Research, FDA

NTP Board of Scientific Counselors Meeting,

June 11-12 2008, Research Triangle Park, NC

Outline

- Furan in food
- Exposure assessment
- Metabolism
- Carcinogenicity
- Regulatory issues
- Proposed study elements
- Outcomes

Formation of Furan in Foods

- Heating - canned and jarred foods; baked/toasted cereal products; coffee
- Volatility
- Irradiation
- Ascorbate
- Carbohydrates ± amino acids
- Peroxidized polyunsaturated fatty acids

Furan Levels Identified by FDA

- Limited variability within tested food types
- Ranges from <LOD to 175 µg/kg
- Jarred infant foods
  - Sweet potatoes and garden vegetables, 15 to 110 µg/kg
  - Other vegetables, 30 to 65 µg/kg
  - Pastas containing meat, 10 to 90 µg /kg
  - Juices, <10 µg/kg
  - Fruits, <30 µg/kg
  - Formula, <LOD to 21 µg/kg (as ready to use)
    - All powdered samples were <LOD

Furan Levels Identified by FDA

- Adult Foods
  - Coffee, <LOD to 80 µg/kg
    - Brewed coffee levels typically higher than instant coffee levels
  - Juices, <LOD to 30 µg/kg
  - Canned or cured meats, <LOD to 40 µg/kg
  - Canned fish, < LOD to 10 µg/kg
  - Snack foods, <LOD to 65 µg/kg
  - Cereals, 10 to 45 µg/kg
  - Canned mixtures (e.g., chili, soup, pasta meals & sauces, baked beans), < LOD to 125 µg/kg
  - Nutritional drinks, <LOD to 175 µg/kg
  - Gravies, 10 to 175 µg/kg

Exposure from Adult Food Types

Adult Food Type	Exposure
Brewed coffee	0.15 µg/kg bw/d
Chili	0.04 µg/kg bw/d
Cereals	0.01 µg/kg bw/d
Salty Snacks	0.01 µg/kg bw/d
Soups containing meat	0.01 µg/kg bw/d
Pork and beans	0.004 µg/kg bw/d
Canned pasta	0.004 µg/kg bw/d
Canned string beans	0.004 µg/kg bw/d
Pasta sauces	0.001 µg/kg bw/d
Juices	0.001 µg/kg bw/d
Canned tuna (in water)	0.00008 µg/kg bw/d

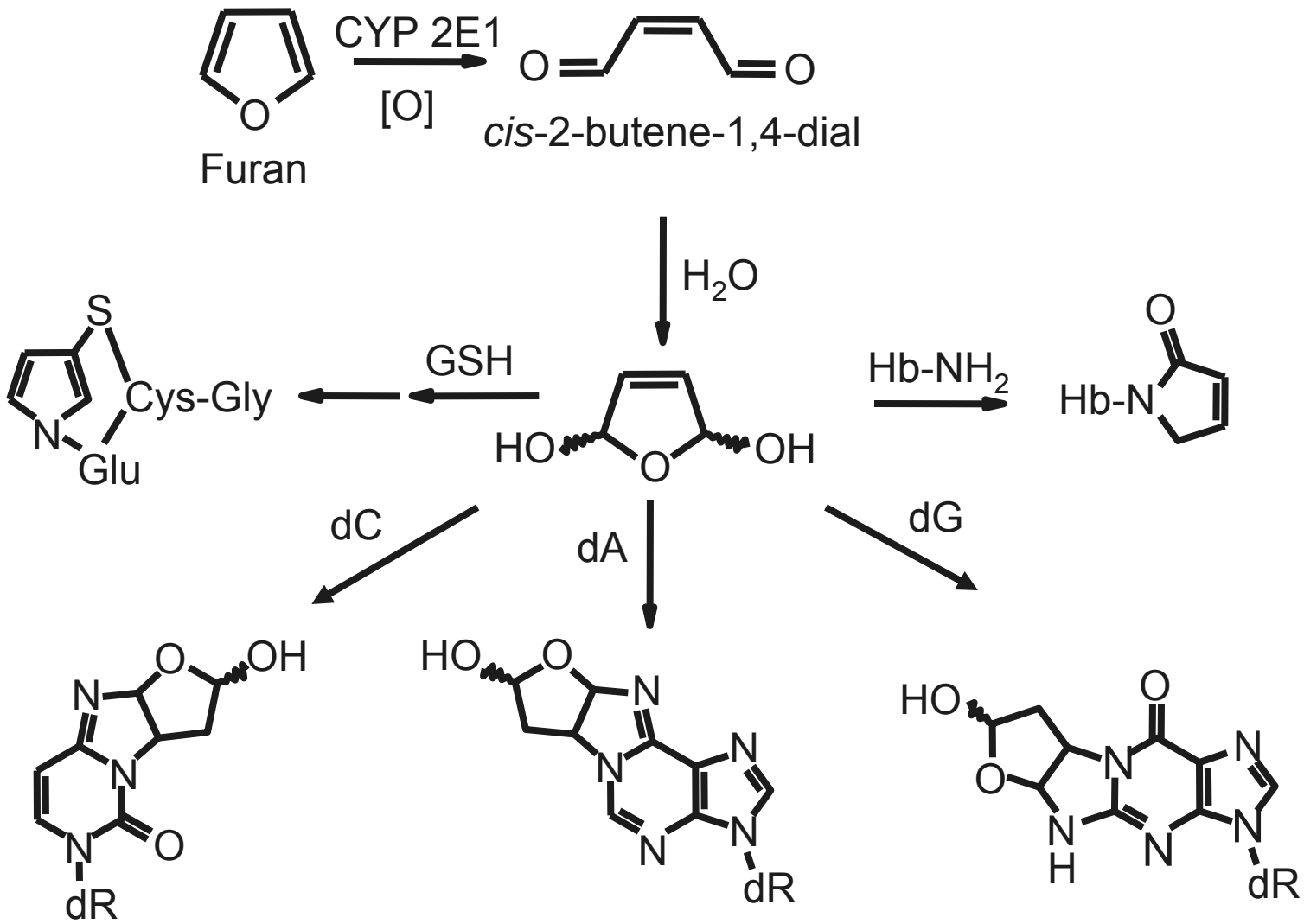
U.S. Dietary Furan Exposure

- Based on current concentration data (Morehouse et al., Fd. Add. Contam. 2008)
- Adult Foods (2+ years old)
  - Mean 0.25 µg/kg bw/day
  - 90th percentile 0.61 µg/kg bw/day
- Infant foods (0-1 years old)
  - Mean 0.41 µg/kg bw/day
  - 90th percentile 0.99 µg/kg bw/day

Furan Exposure

- Infant Formula
  - 100-120 Kcal/kg bw/day needed for growth
  - Formula contains 0.8 Kcal/g
  - Mean furan concentration of 7 µg/kg
- Exposure
  - 0.9 µg/kg bw/day

Furan Oxidative Metabolism and Adduct Formation



Similarities Between Furan and Acrylamide

- Heating-induced formation
- Wide distribution in food supply
- Parent compounds not mutagenic in Salmonella tests
- Reactive oxygenated metabolites are mutagenic
- Activated by CYP 2E1
- Carcinogenic - rodent bioassays
- Some evidence for non-genotoxic mechanisms of carcinogenesis

NTP 2 yr Carcinogenesis Studies -TR 402 (1993)

- Male and female B6C3F1 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

NTP 2 yr 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

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 B6C3F1 liver tumors
  - (C → A transversions, A → G transition)
- 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, necrosis
- Mouse bioassay doses produced:
  - Biliary tract – chronic inflammation, cyst, fibrosis, hyperplasia and metaplasia
  - Hepatocyte – cytomegaly, vacuolization, degeneration, hyperplasia, necrosis

Regulatory Issues

- Establish dose response for cholangiocarcinoma in F344 rats
- Can the dose-response relationships for cancer, genotoxicity (DNA adducts), and cytotoxicity in liver be distinguished?
- Is furan mutagenic in vivo (subchronic)?
- Which DNA adduct(s) are causative?
- Can human exposures to low doses of furan in the diet be related with carcinogenicity in rodents?
- Relationship to other cooking carcinogens?

Proposed Study Elements

- Extend the dose-response range for 2 y furan carcinogenicity in male F344 rats (< 2 mg/kg bw/d gavage)
- Determine dose-response for subchronic (≤1 y) furan hepatotoxicity (Ki67 or PCNA expression, etc) in male F344 rats (e.g., 0, 0.1, 0.25, 0.5, 1, 2, 4 mg/kg bw/d gavage)
- Determine reversibility of hepatotoxicity
- Determine dose-response for furan-DNA adduct formation in liver (< 0.1 mg/kg bw gavage)
- Big Blue rat mutagenesis (2, 8, 16, 30 mg/kg bw/d gavage)
- Determine toxicokinetics and exposure biomarkers (DNA & Hb adducts, urinary metabolites) of furan in male F344 rats form single and repeated dosing (target doses 0.1-1 mg/kg bw/d gavage)
- PBPK modeling to integrate kinetics and biomarkers

Possible Outcomes

- Determine dose response for most sensitive cancer endpoint (rat cholangiocarcinoma)
- Distinguish between genotoxic (mutagenic) and hepatotoxic doses/mechanisms
- Establish biomarkers of furan exposure and bioactivation
- Link rodent hepatocarcinogenicity & exposure biomarkers with human biomarkers for risk assessment
- PBPK/PD modeling