

This document was developed by NIEHS/NTP staff to facilitate internal and external review of a proposed research program prior to designing and conducting toxicology studies. The purpose of the research concept document is to outline the general elements of a research program that would address the specific public health concerns that prompted the nomination of the substance or issue for study. It may also encompass substance-specific studies that address larger public health issues or topics in toxicology. Additional information about the nomination, review, and selection of substances for study by the NTP is provided at *Nominations to the NTP Testing Program* (<http://ntp.niehs.nih.gov/go/nom>). A draft version of this research concept was reviewed by the NTP Board of Scientific Counselors at a public meeting on June 11-12, 2008 (<http://ntp.niehs.nih.gov/go/9741>) and subsequently approved by the NTP Executive Committee.

NTP Research Concept: Furan

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Background

Furan (CASRN: 110-00-9) is formed in many common foods during heating of either ascorbate, carbohydrates (with or without an amino acid), or polyunsaturated fatty acids (Locus and Yaylayan, 2004). Dietary exposure modeling for the U.S. population, based on measured concentrations of furan in numerous foods conducted by CFSAN/FDA (Morehouse et al., 2008), predicts mean consumption of 0.26 µg/kg bw/d furan in adult foods (persons over 2 yr of age), 0.41 µg/kg bw/d in children's food (0-1 yr of age), and 0.9 µg/kg bw/d in infants consuming formula as the primary source of nutrition.

The NTP previously determined that there was clear evidence of carcinogenic activity of furan in male and female F344 rats based on increased incidences of cholangiocarcinoma and hepatocellular neoplasms of the liver and increased incidences of mononuclear cell leukemia in a 2 yr gavage study using oral gavage doses of 2, 4, or 8 mg/kg bw/d; the NTP similarly found clear evidence of carcinogenic activity of furan in male and female B6C3F₁ mice based on increased incidences of hepatocellular neoplasms of the liver using doses of 8 or 15 mg/kg bw/d (NTP, 1993). Rats were more sensitive to the carcinogenic effects of furan than mice, with nearly all dosed males and females showing cholangiocarcinomas versus none in the controls.

The doses used previously for the chronic cancer bioassays also caused cytotoxicity and necrosis in liver, kidney, and other organs. There are other studies demonstrating regenerative cell proliferation secondary to such doses of furan and it is not possible to distinguish dose-response characteristics of genotoxic effects from chemically reactive furan metabolites (e.g., *cis*-2-butene-1,4-dial) from those of compensatory hyperplasia secondary to hepatotoxicity (Wilson et al., 1992).

The recent NTP/NCTR investigations of acrylamide point out notable similarities to the issues surrounding furan: both compounds are carcinogenic in rodent bioassays, even though the location of tumors produced in F344 rats are quite dissimilar between the two compounds (e.g., liver and leukemia for furan vs. CNS, thyroid, peritesticular mesothelium, and mammary for acrylamide; reviewed in Doerge et al., 2008); both compounds are produced in many common foods during normal cooking; while both parent compounds were reported as being non-mutagenic in *Salmonella* testing, both of the reactive oxygenated metabolites are direct acting mutagens (e.g., *cis*-2-butene-1,4-dial and glycidamide, respectively) and form DNA adducts; both compounds are oxygenated in liver primarily by CYP 2E1 (see Scheme 1); and both

genotoxic and non-genotoxic mechanisms of carcinogenicity have been proposed for each compound have evidence for both.

The widespread exposure to furan through the diet is a public health concern because of the demonstrated potential for hepatotoxicity and carcinogenicity observed in two rodent species, particularly the high incidences observed for cholangiocarcinomas in male and female F344 rats in the previous NTP 2 y studies. Despite distinct anatomical differences between rats and humans (i.e., rats have no gall bladder), cholangiocarcinomas also occur in humans and are associated with a very poor prognosis.

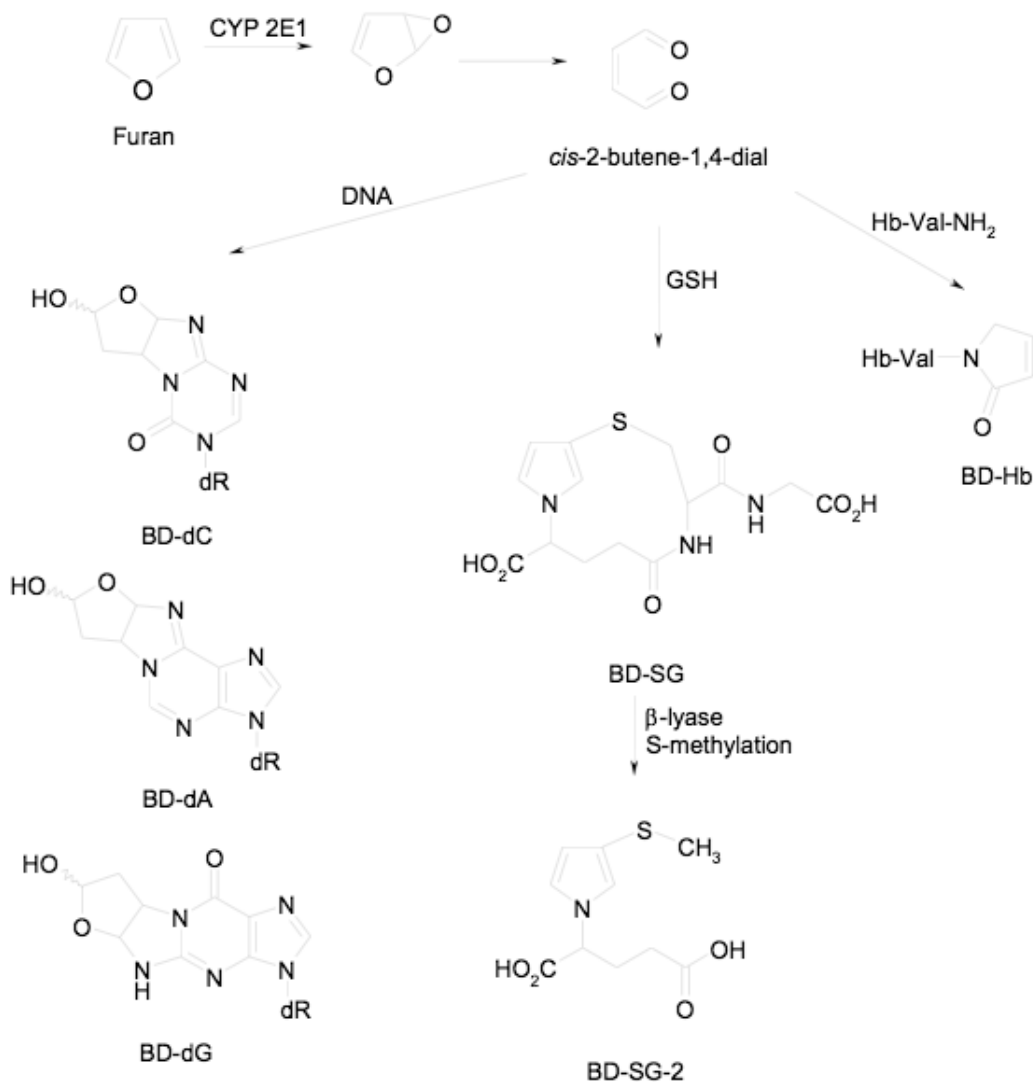


Figure 1. Oxidative metabolism of furan and formation of potential biomarkers.

Key Issues

There are several key issues that need to be addressed in order to understand the human cancer risks associated with furan exposure.

1. All doses of furan used in the previous NTP 2 y study in F344 rats (2, 4, and 8 mg/kg bw/d; National Toxicology Program, 1993) induced quantitative adjusted rates of cholangiocarcinomas. Therefore, no dose response relationship can be ascertained from this the most sensitive indicator of furan-induced carcinogenesis. This data gap needs to be rectified before regulatory risk assessment of furan carcinogenicity can be performed.
2. All doses of furan used in the previous NTP 2 y study in F344 rats (2, 4, and 8mg/kg bw/d; NTP, 1993) also produced significant increases in hepatotoxicity. Therefore it is not possible to understand fully the role for DNA-damaging (genotoxic) effects as opposed to regenerative hyperplasia (non-genotoxic) in the mechanism of furan hepatocarcinogenicity. Mechanistic resolution of this question requires additional information from lower doses, particularly for a) chronic carcinogenicity (see above) b) DNA adduct formation in livers of furan-treated rats, as well as c) indices of hepatotoxicity. The relationships between dose response relationships for these endpoints are important data gaps required for regulatory risk assessment of furan carcinogenicity.
3. Limited information is available to understand the ability of furan to induce mutagenesis *in vivo*, not only from a hazard identification standpoint, but also the dose response and relationship to hepatocarcinogenic doses. The role of mutagenesis as a causative factor in the carcinogenic mechanism is a critical piece of evidence required for regulatory risk assessment of furan.
4. Predictive biomarkers for exposure and bioactivation by been developed, validated, and applied in experimental animal studies in order to estimate risks from acrylamide carcinogenicity in human populations (Doerge et al., 2008). Similar opportunities are possible for furan but additional information is needed about the metabolism of furan through its reactive metabolite, *cis*-2-butene-1,4-dial, from controlled exposures in F344 rats in conjunction with detailed toxicokinetic analysis using doses similar to those used in the modified bioassays (see above). Such results could provide additional kinetic and biomarker data for integration into an existing PBPK model (Kedderis et al., 1993) as a link to future human biomonitoring studies. Information about internal exposures of humans to reactive carcinogen metabolites can be critical evidence for dose and species extrapolations made in regulatory cancer risk assessment.
5. The potential for altered cancer susceptibility following developmental (transplacental and/or lactational transfer) exposure to furan has not been adequately explored. Carcinogen susceptibility can be either increased because of rapid cell proliferation and growth during development (a toxicodynamic effect) or decreased because of lower capacity for metabolic activation carcinogen (a toxicokinetic effect). Information about life stage susceptibility is often a critical component of regulatory cancer risk assessments.

Proposed Approach

1. A 2 yr carcinogenicity study will be conducted in adult F344 rats, extending the dose range lower than the previous NTP study, with some overlap (e.g., \leq the lowest dose of 2 mg/kg bw/d).

The aim of this study will be to more fully evaluate the dose-response for carcinogenicity following furan exposure.

2. To understand better the role of genotoxicity in the carcinogenicity of furan, the dose response characteristics for DNA adduct formation with *cis*-2-butene-1,4-dial, the putative genotoxic furan metabolite, would also be determined in livers from furan-treated rats (single and repeated dosing) using LC/MS/MS techniques (Byrns et al., 2002). In addition, sub-chronic exposure studies (e.g., ≤ 1 month) using lower doses than the previous NTP study would allow determination of the dose response for hepatotoxicity, regenerative hyperplasia, and cell proliferation (e.g., Ki67 and/or PCNA staining) in target liver cells and the reversibility of such effects.

3. The mutagenic potential for furan and dose response will also be assessed by conducting subchronic in vivo mutagenesis assays using Big Blue rats using doses similar to that produced increased liver tumor incidences in the 2 y NTP study (i.e., ≤ 2 mg/kg bw/d). This transgenic rat, genetically derived from F344, contains multiple copies of chromosomally incorporated plasmid and shuttle vectors containing reporter genes for detection of mutations all tissues. Strengths of this assay include the ability to determine increased mutant frequencies of either the transgene in target tissues (e.g., *cII*) and endogenous genes (e. g., *Hprt* in spleen); in addition, sequencing of the mutated transgene can provide information regarding the type(s) of mutations and their relationship with observed DNA adducts (Manjanatha et al., 2006).

4. Toxicokinetic studies using doses of furan relevant to those that produced increased liver tumors in the 2 y NTP study (i.e., ≤ 2 mg/kg bw/d) will be conducted in plasma and liver. In addition to the DNA adducts described above (#2), additional biomarkers of furan exposure and bioactivation would be determined, including adducts between *cis*-2-butene-1,4-dial and either hemoglobin or glutathione in erythrocytes or urine, respectively (Scheme 1). The intravenous and oral routes of administration will be used, including corn oil gavage and possibly some sort of modified food preparation to understand better bioavailability of furan.

5. To understand better the effect of transplacental and/or neonatal exposures to furan, a transplacental arm could be added to #1 in which selected common dose(s) would be administered to pregnant dams throughout lactation and then the weanlings would be chronically exposed for the entire lifetime as described above for #1.

Significance and Expected Outcome

It is important to understand how genotoxicity and mutagenicity contribute to the carcinogenic action of furan in rats separately from cytotoxic effects of furan that induce regenerative hyperplasia. Dose response information for these endpoints will be determined by the proposed research. In addition, it is necessary to extrapolate cancer risks in humans across species (rat to human) and across the doses required to induce increased incidences of liver tumors in reasonable numbers of rats (≤ 2 mg/kg bw/d) as opposed to the low doses to which humans are exposed in the diet (< 1 μ g/kg bw/d). The measurement of liver DNA and hemoglobin adducts with *cis*-2-butene-1,4-dial in rodents exposed to doses of furan that induce cancer and even lower should permit modeling of the relationship between DNA adducts and cancer incidences and with hemoglobin adducts. Developing potential human biomarkers of furan exposure

(hemoglobin adducts, urinary metabolites) could provide the linkage needed to estimate genetic damage (DNA adduct formation) in humans as a way to reduce uncertainty in cancer risk assessment.

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