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Low dose assessment of the carcinogenicity of furan in male F344/N Nctr rats in a 2-year gavage study

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ABSTRACT

Furan is a volatile organic chemical that is a contaminant in many common foods. Furan is hepatocarcinogenic in mice and rats; however, the risk to humans from dietary exposure to furan cannot be estimated accurately because the lowest tested dose of furan in a 2-year bioassay in rats gave nearly a 100% incidence of cholangiocarcinoma. To provide bioassay data that can be used in preparing risk assessments, the carcinogenicity of furan was determined in male F344/N Nctr rats administered 0, 0.02, 0.044, 0.092, 0.2, 0.44, 0.92, and 2 mg furan/kg body weight (BW) by gavage 5 days/week for 2 years. Exposure to furan was associated with the development of malignant mesothelioma on membranes surrounding the epididymis and on the testicular tunics, with the increase being significant at 2 mg furan/kg BW. There was also a dose-related increase in the incidence of mononuclear cell leukemia, with the increase in incidence being significant at 0.092, 0.2, 0.92, and 2 mg furan/kg BW. Dose-related non-neoplastic liver lesions included cholangiofibrosis, mixed cell foci, basophilic foci, biliary tract hyperplasia, oval cell hyperplasia, regenerative hyperplasia, and cytoplasmic vacuolization. The most sensitive non-neoplastic lesion was cholangiofibrosis, the frequency of which increased significantly at 0.2 mg furan/kg BW.

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1. Introduction

Furan (Fig. 1) is a volatile organic chemical used as a synthetic intermediate and in the production of pesticides, stabilizers, and pharmaceuticals (International Agency for Research on Cancer, 1995; Hazardous Substances Data Bank, 2011). The major sources of exposure for the general public to furan are tobacco products and food. Mainstream cigarette smoke is estimated to contain up to 65 μg furan/cigarette (Smith et al., 2000; International Agency for Research on Cancer, 2004). Furan is produced during the cooking of many common foods, including coffee, baked or fried cereal products, canned and jarred foods, baby food, and infant formula (Hasnip et al., 2006; Nyman et al., 2006; Zoller et al., 2007; Morehouse et al., 2008). The mean daily dietary consumption of furan in the U.S. has been estimated to be 0.25 μg/kg body weight (BW) in adults (Morehouse et al., 2008), 0.41 μg/kg BW in children during their first year of life, and 0.9 μg/kg BW in infants consuming only formula (DiNovi and Mihalov, 2007). Adults in Europe have been estimated to have a median dietary exposure to furan of 0.78 μg/kg BW (European Food Safety Authority, 2009). Coffee contributes approximately 50% of the total dietary exposure of adults to furan in the U.S. (Morehouse et al., 2008).

Abbreviations: BMD, benchmark dose; BMDL, lower limit of benchmark dose; BW, body weight; FDA, Food and Drug Administration; GLP, Good Laboratory Practice; MOE, margin of exposure; NCTR, National Center for Toxicological Research; NIEHS, National Institute of Environmental Health Sciences; NIH, National Institutes of Health; NTP, National Toxicology Program.

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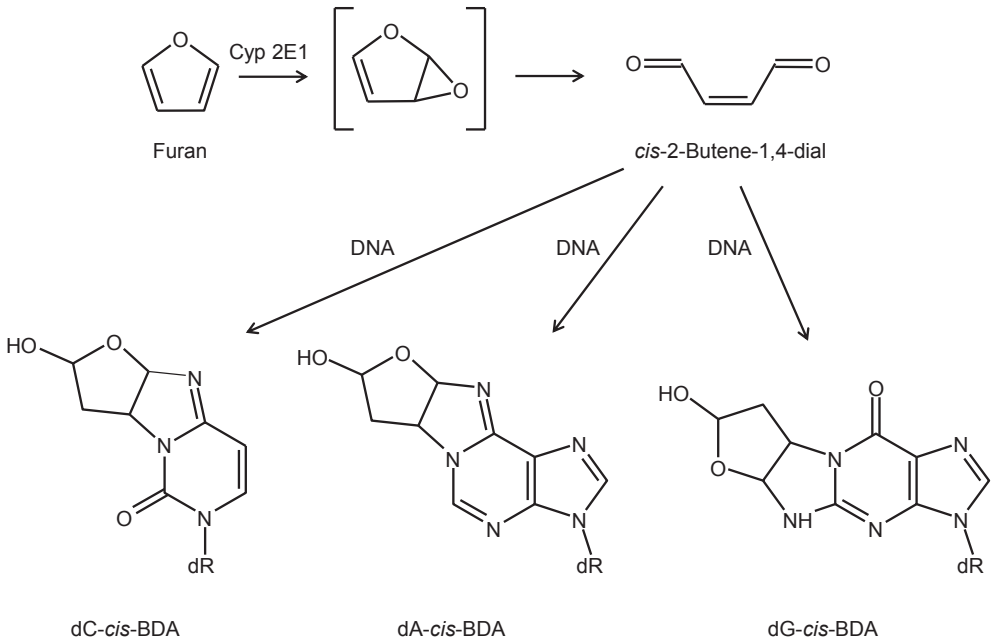

Fig. 1. Structures of furan, *cis*-2-butene-1,4-dial, and the DNA adducts resulting from reaction of *cis*-2-butene-1,4-dial with DNA. The abbreviations used are: Cyp 2E1, cytochrome P450 2E1; dC, deoxycytidine; dA, deoxyadenosine; dG, deoxyguanosine; dR, deoxyribose; *cis*-BDA, *cis*-2-butene-1,4-dial.

Furan is rapidly absorbed from the gastro-intestinal tract and extensively metabolized, primarily by hepatic cytochrome P450 2E1, to *cis*-2-butene-1,4-dial (Fig. 1; Kedderis et al., 1993; Chen et al., 1995), a highly reactive metabolite that can react with thiol and amino groups in glutathione and other peptides (Chen et al., 1997; Peterson et al., 2006; Kellert et al., 2008a; Lu and Peterson, 2010). In addition to forming amino acid adducts, *cis*-2-butene-1,4-dial can react with the exocyclic nitrogens of deoxycytidine, deoxyadenosine, and deoxyguanosine to form bicyclic hemiketal derivatives (Fig. 1; Gingipalli and Dedon, 2001; Byrns et al., 2002; Bohnert et al., 2004). The deoxyadenosine and deoxyguanosine adducts are relatively unstable and undergo ring-opening and dehydration to form secondary etheno-type DNA adducts (Byrns et al., 2004). By using *O*-benzylhydroxylamine as a trapping agent, the deoxyadenosine and deoxycytidine adducts were detected in DNA reacted with *cis*-2-butene-1,4-dial (Byrns et al., 2006). Accelerator mass spectrometry was used to assess the formation of *cis*-2-butene-1,4-dial deoxynucleoside adducts in liver DNA from rats administered 0, 0.1, or 2 mg [3,4-^{14}C]furan/kg BW. There was a dose-related increase in ^{14}C associated with liver DNA; however, upon hydrolysis and chromatography of the DNA, only very limited amounts of the radioactivity corresponded to the previously characterized *cis*-2-butene-1,4-dial deoxynucleoside adducts (Neuwirth et al., 2012). More recently, the presence of deoxycytidine *cis*-2-butene-1,4-dial, the major and most stable of the DNA adducts obtained from furan, was examined in the livers of F344 rats treated 5 days/week by gavage for up to 360 days with 4.4 mg furan/kg BW (Churchwell et al., 2015). Formation of DNA adducts was not detected above the background level of 1–2 adducts/10^8 nucleotides.

The carcinogenicity of furan has been assessed in mice and rats. In male and female B6C3F$_1$ mice treated by gavage 5 days/week with 0, 8, or 15 mg furan/kg BW for 2 years, there was a dose-dependent increase in hepatocellular adenoma, carcinoma, and combined adenoma or carcinoma. With the exception of hepatocellular carcinoma in female mice, the incidence of each of these neoplasms was significantly increased at both doses of furan (National Toxicology Program, 1993). In a subsequent study, female B6C3F$_1$ mice were administered 0, 0.5, 1, 2, 4, or 8 mg furan/kg BW 5 days/week for 2 years. There was a dose-dependent increase in hepatocellular adenoma, carcinoma, and combined adenoma or carcinoma, with the incidence of adenoma and combined adenoma or carcinoma being significant at 4 and 8 mg furan/kg BW and the incidence of carcinoma being significant at 8 mg furan/kg BW (Moser et al., 2009). The carcinogenicity of furan has also been assessed in mice treated as newborns. Male B6C3F$_1$ mice given a single intraperitoneal injection of 400 mg furan/kg BW on postnatal day 15 had a significant increase in liver adenoma. Likewise, male B6C3F$_1$ mice given intraperitoneal injections of 200 mg furan/kg BW on postnatal days 3, 6, 9, 12, 15, and 18 had a significant increase in liver adenoma and combined liver adenoma or carcinoma (Johansson et al., 1997).

In male and female F344/N rats treated by gavage with 0, 2, 4, or 8 mg furan/kg BW 5 days/week for 2 years there was a dose-dependent increase in hepatocellular adenoma, carcinoma, and combined adenoma or carcinoma, liver cholangiocarcinoma, and mononuclear cell leukemia in both sexes. The incidence of each of the neoplasms, with the exception of hepatocellular carcinoma in female rats, was significantly increased at 4 and 8 mg furan/kg BW; liver cholangiocarcinoma was also significantly increased at 2 mg furan/kg BW. Likewise, the incidence of liver cholangiocarcinoma was significantly elevated in male and female rats treated by gavage with 2, 4, or 8 mg furan/kg BW 5 days/week for 9 or 15 months. In a subsequent experiment, male F344/N rats were administered by gavage 30 mg furan/kg BW 5 days/week for 13 weeks. When subgroups were assessed at the end of dosing and at 9 and 15 months after the initiation of dosing, the incidence of liver cholangiocarcinoma was 0, 100, and 100%, respectively (National Toxicology Program, 1993).

Although furan is carcinogenic in mice and rats (with rats being more sensitive than mice), the risk to humans from dietary exposure to furan cannot be estimated accurately because the lowest tested dose of furan (2 mg/kg BW) in the 2-year bioassay conducted by the National Toxicology Program (NTP) in rats gave nearly a 100%