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A 90-day subchronic gavage toxicity study in *Fischer* 344 rats with 3-methylfuran

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ABSTRACT

A 90-day gavage study was conducted with 0.0, 0.02, 0.075, 0.25, 1.0 and 4.0 mg/kg bw/day dose groups of 3-methylfuran to identify a no-observed adverse effect level for hepatotoxicity and to characterize non-neoplastic effects including changes in gross anatomy, histopathology, clinical biochemistry and hematology. There were significant changes in the serum clinical biochemistry markers related to liver injury where males were more affected than the females for most parameters analysed. The serum liver injury marker γ−glutamyltransferase, alanine and aspartate aminotransferases were significantly increased in males in the 4.0 mg/kg dose group. Alkaline phosphatase was increased in females and males. There were increases in both gross and histological lesions in the liver of both sexes in addition to statistical differences in female liver weights at the 4.0 mg/kg bw/day dose. Significant increases in spleen weights were found in both genders. This was accompanied by a dose–dependent atrophy of both B- and T-cell regions in which the males were more affected. There were no significant changes in male kidney weights but there was microscopically decreased protein in the proximal tubules and crowding of their nuclei in the 4.0 mg/kg bw/day dose group. There were also significant changes in the kidney serum biomarkers including various electrolytes, blood urea nitrogen, creatinine and uric acid. A small, but significant increase in female kidney weights was observed and which increase was accompanied by changes in electrolytes, kidney specific markers and a dose-dependent increase in mineralization. In both genders, amylase decreased whereas lipase increased but these were not accompanied by any histological changes in the pancreas.

Histopathological changes in the liver were observed consistently in male and female rats in the 0.25 mg/kg dose group and higher. Hence, a lowest observed adverse effect level (LOAEL) of 0.25 mg/kg bw/d and a no observed adverse effect level (NOAEL) of 0.075 mg/kg bw/day are proposed for 3-methylfuran-induced hepatic lesions in this study. Benchmark dose modelling based on a BMR of 10% change in lesion incidence, generated $BMDL_{s10}$ of 0.08 mg/kg bw/day in male rats and 0.05–0.17 mg/kg bw/day in female rats for increased incidence of liver lesions.

1. Introduction

Furan is a substance that can form naturally in certain foods as a result of heating and has been detected in a wide variety of foods, notably those packaged in cans or jars (Maga, 1979). Furan has been shown to be a potent hepatotoxin and hepatocarcinogen in experimental animals (NTP, 1993; Gill et al., 2010). Furan treatment can also induce toxicity in the reproductive system of male rats from weaning through post puberty (Karacaoğlu and Selmanoğlu, 2010). Based on these results the International Agency for Research on Cancer (IARC, 1995) has classified furan as “possibly carcinogenic to humans” (group 2B). The harmful effects of furan results from the cytochrome P450 catalyzed furan ring oxidation which yields a reactive *cis*-butene-1,4-dial metabolite, which in turn can bind to various cellular components, including protein and DNA (Peterson, 2013).

Surveys to measure the levels of furan in food and estimate dietary exposure to furan have been conducted by various international agencies and between 2004 and 2010 the analysis of furan content in food included a total of 5050 analytical results submitted by 20 countries (Seok et al., 2015; EFSA, 2011). These foods included cooked and canned meats, roasted coffee, beer and bakery products such as toasted bread and rusks (Huault et al., 2016; Bolger et al., 2009; Crew and

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List of abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	One-way analysis of variance
AST	Aspartate aminotransferase
BMD	Benchmark Dosing
BUN	Blood urea nitrogen
BW	Body weight
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GTT	γ−glutamyl transferase
H&E	Hematoxylin and eosin
HCT	Hematocrit
HGB	Hemoglobin
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
NOAEL	No observed adverse effect level
NBF	Neutral buffered formalin
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
PLT	Platelets
RBC	Red blood cell count
RDW	Red blood cell distribution width
v:w	Volume-to-weight
WBC	White blood cell count

Castle, 2007; Maga, 1979; Limacher et al., 2007, 2008; Morehouse et al., 2008; Becalski et al., 2010; 2016). Some furan derivatives may also be used as flavouring agents in some foods and tobacco products (Maga, 1979). The derivative, 3-methylfuran, does not appear to be used to directly add flavour to foods.

In most thermally treated products, the parent chemical furan has been found to be accompanied by a series of alkylated analogues, in particular mono-substituted alkylfurans, such as 2-methyl, 3-methyl, and 2-ethylfuran (Maga, 1979; Mark et al., 2006; Becalski et al., 2010; 2016). Previous surveys on furan, 2- and 3- methylfurans in foods indicated that coffee might be an important source of methylfurans in the diet (Becalski et al., 2010, 2016; Fromberg et al., 2014). Alkylfurans were shown to form in model reactions similar to furan using precursors commonly found in foods (Adams et al., 2011; Becalski and Seaman, 2005; Limacher et al., 2008). The 2- and 3-methyl substituted furans are metabolically activated in a similar fashion as the parent furan yielding α, β-unsaturated dialdehydes/aldehyde ketones (Ravindranath et al., 1984). Previous surveys of levels of furan found in foods sold in Canada (Becalski et al., 2010; 2016) have shown that the levels of methylfurans in jarred food approach 50% of the concentration of furan. The levels of 2-methylfuran in ground coffee were estimated to be twice those of furan (Becalski et al., 2010, 2016). Deterministic exposures to furan and total furan from the diet by adults (greater than 20 years) were, on average, 0.37 and 0.71 μg/kg bw per day, respectively. In the case of toddlers (1–4 years), mean furan and total furan exposures were estimated to be 1.12 and 1.34 μg/kg bw per day, respectively (Becalski et al., 2010; Health Canada, 2016). These data agree with the data collected by the World Health Organization (WHO, 2011).

Until recently, little attention had been paid to the effects and presence of methylfurans in foods until 2-methylfuran and 3-methylfuran were identified to be potent hepatotoxins (Wiley et al., 1984; Ravindranath et al., 1984; Ravindranath and Boyd, 1991). Little toxicological information was available for 3-methylfuran and in most of the available studies, the route of administration was by inhalation (Haschek et al., 1983;1984; Morse et al., 1984; Gammal et al., 1984) or intraperitoneal injection. With inhalation studies, furan, 2-methylfuran and 3-methylfuran were found to induce pulmonary, nasal and kidney toxicity (Gammal et al., 1984; Haschek et al., 1983;1984; Morse et al., 1984; Ravindranath et al., 1984; Witschi et al., 1985). 2-methylfuran and 3-methylfuran have also been found to cause centrilobular necrosis of the liver as well as pulmonary bronchiolar lesions in mice (Wiley et al., 1984; Walinder et al., 1998). These are activated by rat microsomal systems (Ravindranath et al., 1984; Haschek et al., 1984) and metabolites of radiolabeled 3-methylfuran administered intraperitoneally can bind to both DNA and protein (Ravindranath et al., 1984). While inhaled 3-methylfuran appears to target the same organs as inhaled 2-methylfuran, less information is available on the effects of 3-methylfuran with other routes of administration and on its mechanisms of action.

Previously we have conducted independent gavage studies in rats using 2- and 3-methylfuran (28 days) and furan (90 days) to study toxicological effects through an oral route of exposure (Gill et al., 2010, 2014, 2015) which resulted in the liver being identified as the main target organ. However, there are no available toxicology studies in rodents identifying a No Observed Adverse Effect Level (NOAEL) for either 2-methylfuran or 3-methylfuran. In the 28-day studies, the two methyl furans (MF) were not tested at the two lowest dose that was used in the 13-week study with furan (0.03 mg/kg bw/day), and in the case of 2-MF, it was not tested at 0.1 or 0.3 mg/kg bw/day either. Despite the shorter duration of the 3-MF study, gross changes in the liver were first observed at a lower dose with 3-MF (3 mg/kg bw/day) than with furan (8 mg/kg bw/day). No gross changes were seen with furan at 2 mg/kg bw/day, the closest dose to 3 mg/kg bw/day in that study. Histological changes (e.g., Kupfer cells with pigment, apoptosis of hepatocytes) that were evident at the low dose of furan (0.12 mg/kg bw/day) at 3-months were also evident at the low dose (0.1 mg/kg bw/day) of 3-MF at 28 days. These histological changes were not seen with furan at 0.03 mg/kg bw/day. While the dose at which histological changes were seen was higher in 2-MF than in 3-MF (1.5 vs. 0.1 mg/kg bw/day), increases in liver weights occurred at a lower dose with 2-MF than with 3-MF (3 vs 12 mg/kg bw/day). Overall, these data are suggestive that 3-MF is more toxic than either furan a or 2-MF. The research on the human health risk of 3- methylfuran in food is needed to fully understand if levels in food pose a health risk, and if there are any measures that can be taken to minimize or reduce quantities present. To address this data gap, a rodent 90-day subchronic study using low doses of 3-methylfuran was conducted to better characterize effects on hepatotoxicity, hematology, clinical biochemistry, gross morphology and histopathology parameters.

2. Materials and methods

2.1. Test compound and dosing solutions

3-methylfuran (Sigma-Aldrich, USA, purity > 95%) was mixed with Mazola® corn oil to deliver final doses of 0.0 0.02, 0.075, 0.25, 1.0 and 4.0 mg/kg bw/day. Each dose was prepared separately on a volume-to-weight (v:w) ratio. Chilled corn oil was weighed to the nearest milligram in a conical flask. Chilled furan solution was drawn up in a Hamilton syringe, measured to the nearest microliter, injected into the corn oil and mixed using a magnetic stir bar. Dosing solutions were dispensed into brown glass vials and capped with plastic closures adapted with silicon septa. Dosing solutions were stored in a refrigerator at 4°C. Fresh solutions were prepared every 14 days. Previous studies have demonstrated the stability of furan dosing formulations for at least 14 days under these conditions (NTP, 1993).