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Furan is not genotoxic in the micronucleus assay *in vivo* or *in vitro*

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Abstract

Furan, a potential human carcinogen, is formed during heat-treatment of food. Previous studies of the genotoxicity of furan have given disparate results. Hence, there is a need for complementary data to clarify the mechanism behind the carcinogenicity of furan. In this study, we have used the flow cytometer-based micronucleus assay in mice and the cytokinesis-block micronucleus assay in human lymphocytes to investigate the genotoxic potential of furan. Three *in vivo* experiments were performed: intraperitoneal or subcutaneous injection of furan in male Balb/C mice (0–300 and 0–275 mg/kg body weight, respectively) and intraperitoneal injection of male CBA mice (0 and 225 mg/kg body weight). No increased level of micronucleated erythrocytes was detected in any of the *in vivo* experiments. In the *in vitro* setup, human lymphocytes from two donors were treated with furan in concentrations from 0 to 100 mM, either with or without metabolic activation (liver homogenate from rat). In parity with the *in vivo* results there was no significant increase in the frequency of micronucleated cells here either. As neither the *in vivo* nor the *in vitro* studies disclose any significant increase in the micronucleus frequency after treatment with furan, our results support that the carcinogenicity of furan is caused by a non-genotoxic mechanism.

Keywords: Furan; Micronucleus assay; Flow cytometer; Chromosome aberration

1. Introduction

Heat-induced food toxicants have during the last years reemerged as a topic of interest. This is especially true after it was reported that acrylamide is formed during heat treatment of carbohydrate-rich foods (Tareke et al., 2002; Svensson et al., 2003). However, a great variety of other, possibly harmful, compounds are formed during the heating process. One such compound is furan that has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, 1995).

It has since long been known that the heterocyclic compound furan is present in some kinds of food as a flavour volatile (Maga, 1979). In 2004, the US Food and Drug Administration (FDA) performed a survey and found that furan was present in numerous kinds of heat-treated foods (FDA, 2005). The highest levels were found in canned and jarred products like baby food, soups, sauces, vegetables, and fruits, but furan is also found in other products, for example in coffee (FDA, 2005). The mechanism behind furan formation in food is still not fully understood. One proposed pathway is thermal degradation or rearrangement of organic compounds, particularly carbohydrates (Maga, 1979), but

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there are probably multiple routes of formation. Besides the occurrence of furan in food it also has a wide use as an intermediate and solvent in industrial processes. Despite this extensive use, information on the toxicology of furan is still incomplete. After the discovery that furan is present in a broad range of food items, it is even more important to clarify the toxicological properties of it.

The mechanism behind the carcinogenic effect of furan is not well understood. Various studies suggest both genotoxic (Stich et al., 1981; McGregor et al., 1988; NTP, 1993) and non-genotoxic mechanisms (Mortelmans et al., 1986; Wilson et al., 1992; Fransson-Steen et al., 1997). Furan is metabolised into the key metabolite, *cis*-2-butene-1,4-dial, by CYP2E1. This metabolite is probably responsible for the carcinogenic properties of furan (Byrns et al., 2006). It is both cytotoxic and binds to proteins (Burka et al., 1991) and nucleosides (Byrns et al., 2002).

Genotoxic activity has an impact on the risk assessment of a chemical. Therefore, it is important to further investigate these properties of furan, as the results from bacterial culture and whole animal genotoxicity studies are disparate. European Food Safety Authority (EFSA) (2004) and FDA (2004) have asked for complementary studies to clarify this matter, to be able to perform a thorough risk assessment of furan. Furthermore, to determine if furan in food constitutes a health risk, additional toxicological intake data are needed.

A commonly used method to assess the potential of a chemical to induce chromosomal aberrations *in vivo* is the micronucleus assay in mice (e.g. semicarbazide (Abramsson-Zetterberg and Svensson, 2005) and acrylamide (Abramsson-Zetterberg, 2003)). This method allows the detection of both clastogenic (chromatide/chromosome breakage) and aneugenic (chromosome maldistribution) effects. To increase the sensitivity of this method the cells can be scored with a flow cytometer, which gives the opportunity to score a large number of cells. It has been reported that furan is rapidly transported from the peripheral blood system to the liver (Burka et al., 1991). Thus, it is especially important to use sensitive methods when analysing effects of furan, as the bone marrow dose might be low. Another common method is the *in vitro* cytokinesis-block micronucleus assay in human lymphocytes. This method is a way to ascertain that cells are exposed to the test substance.

In the present study, we investigated whether furan increases the micronucleus frequency, both *in vivo* and *in vitro*. This is an important step on the way to discriminate between genotoxic and non-genotoxic mechanisms behind the cancerogenicity of furan.

2. Materials and methods

2.1. Micronucleus assay in vivo in mice (experiment 1)

2.1.1. Animals

Male Balb/C and male CBA mice, 6–7 weeks old, weighing approximately 25 g, were obtained from Scanbur AB, Sollentuna, Sweden. The animals were housed at the National Food Administration in Sweden in a 12 h light/12 h dark cycle with free access to solid food and tap water. All mice were acclimatised 1 week before treatment. The experiment was reviewed and approved by Uppsala Ethical Committee on Animal Experiments, application C228/3.

2.1.2. Chemicals

Furan (CAS no. 110-00-9), Colchicine (CAS no. 64-86-8), and Hoechst 33342 (HO342) was purchased from Sigma–Aldrich, Sweden; Percoll from Amersham Biosciences, Sweden; Fluothane from Astra, Sweden; Thiazole Orange (TO) from Molecular Probes, OR, USA.

2.1.3. Experimental design and sampling

The genotoxic effect of furan was determined using the flow cytometer-based micronucleus assay in mice. The animals were randomly divided into different dose-groups. Three separate experiments were performed (1a–1c). In order to disclose any methodological differences between species two strains of mice, Balb/C and CBA, were used. In the first (1a) and second experiment (1b) Balb/C mice were injected with furan intraperitoneally (i.p.) and subcutaneously (s.c.), respectively. In the third experiment (1c) CBA mice were injected i.p. In all experiments the mice were injected with a single dose of 10 μl/g b.w. Furan was diluted in corn oil just prior to the injection. The positive control mice received injections of 1 mg/kg b.w. colchicine dissolved in PBS.

Forty-two hours after injection, the animals were anaesthetised with Fluothane and blood samples were drawn from the orbital vein into heparinised tubes. Directly after blood sampling the animals were killed by cervical dislocation. The sampling time was based on the results of Cao et al. (1993) and on the knowledge of time between appearance of polychromatic erythrocytes (PCE) in the bone marrow and in peripheral blood (Abramsson-Zetterberg et al., 1996).

In experiment 1a, i.p. administration of furan to male Balb/C mice, a total of 27 mice were used. They were given the following doses of furan: 0, 50, 75, 90, 110, 125, 150, 175, 200, 250, and 300 mg/kg b.w. All groups consisted of two mice except the ones given a dose of 0 and 300 mg/kg b.w., and the positive control (colchicine), which consisted of three mice each.

Experiment 1b, s.c. administration of furan to male Balb/C mice, involved 16 mice, which were given three different doses of furan: 0, 150, and 275 mg/kg b.w. Five mice were given 150 mg/kg b.w. and six mice 275 mg/kg b.w. Three mice were given 0 mg/kg b.w. furan and two mice the positive control colchicine.