Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment of furan exposure in the Norwegian population

Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Contaminants of the Norwegian Scientific Committee for Food Safety

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Trine Husøy (Chair)
Augustine Arukwe
Mona-Lise Binderup
Anne Lise Brantsæter
Christiane Kruse Fæste
Ragna Bogen Hetland

Contributors

Persons working for VKM, either as appointed members of the Committee or as *ad hoc* experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The members of the working group are:

VKM members
Trine Husøy, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics (Chair)

Augustine Arukwe, Panel on Contaminants

Mona-Lise Binderup, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics

Anne Lise Brantsæter, Panel on Contaminants

Christiane Kruse Fæste, Panel on Contaminants

Ragna Bogen Hetland, Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics

Assessed by
The report from the working group has been evaluated and approved by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Contaminants of VKM.

Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics:
Inger-Lise Steffensen (Chair), Jan Alexander, Mona-Lise Binderup, Knut Helkås Dahl, Berit Granum, Ragna Bogen Hetland, Trine Husøy, Jan Erik Paulsen, Tore Sanner, Vibeke Thrane.

Panel on Contaminants:
Janneche Utne Skåre (Chair), Heidi Amlund, Augustine Arukwe, Anne Lise Brantsæter, Gunnar Sundstøl Eriksen, Christiane Kruse Fæste, Kari Grave, Helle Katrine Knutsen, Anders Ruus, Cathrine Thomsen.

Scientific coordinators from the secretariat:
Tor Øystein Fotland, Edel Holene, Inger Therese L. Lillegaard.

Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has on request of The Norwegian Food Safety Authority performed a risk assessment of furan intake in the Norwegian population based on the most recent national food consumption surveys. National occurrence data of furan concentrations in food were preferentially used in the risk assessment. When national data were lacking, VKM has used occurrence data of furan from other countries. The assessment has been performed by the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the VKM Panel on Contaminants.

Furan is a volatile and lipophilic compound formed in a variety of heat-treated commercial foods and contributes to the sensory properties of the product. The substance has been found in a number of foods such as coffee, canned and jarred foods including baby food containing meat and various vegetables. High concentrations of furan have been found in coffee and the presence of furan in jarred baby food and infant formulae has received much attention since such products may be the sole diet for many infants. The occurrence of furan in a variety of foods suggests that there are multiple routes of furan formation rather than a single mechanism.

The Norwegian Food Safety Authority has in 2008 and 2009 collected data on furan concentrations in different food products sold on the Norwegian market (Norwegian Food Safety Authority, 2008). In 2011, the Norwegian Food Safety Authority also decided to analyse commercial porridges for infants and children sold on the Norwegian market, to see if furan could be detected in such products.

The calculated furan exposures from food and beverages are based on data from the nationally representative food consumption surveys; Spedkost, Småbarnskost, Ungkost and Norkost. The consumption for each relevant food or food category in the dietary surveys were multiplied with the corresponding mean furan concentrations and totalled for each individual.

The liver is the main target organ for furan toxicity both in mice and rats, but the rat is the most sensitive species. A dose-dependent increase in hepatocellular adenomas and carcinomas was observed in mice and rats, and an increase in the incidence of cholangiocarcinomas was observed in rat liver. Cholangiocarcinomas in male and female rats were the most sensitive toxicological end point observed in rodents. On the basis of the available data, VKM considers that rat cholangiocarcinomas may be relevant for assessing human risk from furan.

Available *in vivo* data with furan indicate that a reactive metabolite, most likely *cis*-2-butene-1,4-dial (BDA), is formed and that this metabolite can react with DNA and induce mutations. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed, but BDA was found to be genotoxic in several *in vitro* tests. VKM therefore considers that a genotoxic mechanism in furan-induced carcinogenesis cannot be excluded and the substance was assessed as a genotoxic carcinogen.

VKM used the Margin of Exposure (MOE) approach in this risk assessment. The suitability of different studies on cholangiocarcinomas for dose-response modelling was considered. The 9-month interim evaluation of a 2-year study from NTP (1993) was chosen because it demonstrates a dose-response relationship. From this study, a point of departure of 0.02 mg/kg bw/day was chosen, based on a benchmark dose lower bound ($BMDL_{10}$) of 0.14 mg furan/kg bw/day and a correction factor of 7 for shorter than full life-time (2 years) study duration.

For 6-, 12- and 24-month-old children, the main source of furan exposure is jarred baby food. For 4-, 9- and 13-year-old children, the major food source to the furan exposure is breakfast cereals. In adults, the major contribution to the furan exposure is coffee. The highest furan exposure was calculated for 12-month-old infants and ranged from 0.62-1.51 µg/kg bw/day. In adults the furan exposure ranged from 0.27-0.82 µg/kg bw/day.

For mean exposure among infants, children and adolescents, the MOE-values ranged from 29 in 12-month-infants to 2000 in the 13-year-old adolescents. Among high consumers in these groups, the MOE-values ranged from 13 to 400. In adults, the corresponding MOE-values ranged from 59 to 74 for mean furan exposure and from 24 to 26 for high exposure.

It should be noted that this risk assessment of furan contains notable uncertainties and limitations. The use of the 9-month interim study in rats including a correction factor of 7 to derive a point of departure, instead of a full life-time study (2-year) study, likely overestimates the hazard of furan. A possible over-diagnosis of the cholangiocarcinomas, due to the similarities in histopathology between cholangiofibrosis and cholangiocarcinomas in rats, may overestimate the hazard. There are also limitations in assessing food consumption and furan content in foods, leading to uncertainties in estimation of furan exposure.

VKM considers that the current exposure to furan in all age groups, particularly among infants and children, is of health concern.

Key words

Furan, risk assessment, intake, BMD calculations, cancer, genotoxicity

Norsk sammendrag

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet utført en risikovurdering av furaninntak i den norske befolkningen basert på de nyeste nasjonale kostholdsundersøkelsene. I risikovurderingen er det fortrinnsvis brukt nasjonale forekomstdata av furan i mat. VKM har brukt forekomstdata fra andre land der nasjonale data har manglet. Vurderingen er utarbeidet av Faggruppen for tilsetningsstoffer, aroma, matemballasje og kosmetikk og Faggruppen for forurensninger, naturlige toksiner og medisinrester.

Furan er et lettfordamplig og fettløselig stoff som dannes i en rekke varmebehandlede kommersielle matvarer og bidrar til produktenes sensoriske egenskaper. Furan er påvist i mange typer mat, f.eks. kaffe, hermetikk og forseglede matvarer på glass inkludert barnemat som inneholder kjøtt og ulike grønnsaker. Høye konsentrasjoner av furan er funnet i kaffe. Funn av furan i barnemat på glass og i morsmelkerstatning har fått mye oppmerksomhet fordi slike produkter kan være den eneste dietten for mange spedbarn. Furan er påvist i en rekke matvarer og derfor er sannsynligvis flere enn en mekanisme involvert i dannelsen av stoffet.

I 2008 og 2009 foretok Mattilsynet en begrenset kartlegging av furannivå i matvarer på det norske markedet. Mattilsynet bestemte seg i 2011 for også å undersøke kommersielle barnegrøter på det norske markedet, for å se om furan kunne påvises i slike produkter.

Konsumdata fra de nasjonale kostholdsundersøkelsene Spedkost, Småbarnskost, Ungkost og Norkost er brukt til å beregne furaneksponering fra mat og drikke. Konsum av hver relevant matvare eller matkategori i kostholdsundersøkelser ble multiplisert med gjennomsnittlig furankonsentrasjon i matvaren og summert for hvert individ.

Leveren er det viktigste organet for furantoksisitet både hos mus og rotter, men rotte er den mest sensitive arten. En doseavhengig økning av hepatocellulært adenom og karsinom (leverkreft) ble sett hos mus og rotter, og en økning i forekomsten av kolangiokarsinom (gallegangskreft) ble sett i rottelever. Det mest sensitive toksikologiske endepunktet hos gnagere var kolangiokarsinom hos hann- og hunnrotter. Ut i fra tilgjengelige data, mener VKM at kolangiokarsinom hos rotte kan være relevant for å vurdere human risiko av furan.

Tilgjengelige *in vivo* data indikerer at furan danner en reaktiv metabolitt, mest sannsynlig cis-2-buten-1,4-dial (BDA), og at denne metabolitten kan reagere med DNA og indusere mutasjoner. VKM kjenner ikke til at det er utført *in vivo* studier av gentoksisitet av BDA, men BDA er funnet å være gentoksisk i flere *in vitro* tester. VKM mener derfor at en gentoksisk mekanisme ikke kan utelukkes i furanindusert kreft og stoffet er derfor blitt vurdert som et gentoksisk karsinogen.

VKM har brukt metoden ”eksponeringsmargin” (Margin of exposure, MOE) i denne risikovurderingen. Flere studier ble vurdert for å finne den best egnede for dose-responsmodelleringen av kolangiokarsinom. En 9-måneders interimevaluering av NTPs 2-årige rottestudie (1993) ble valgt, fordi den viste en dose-responssammenheng med furaneksponering. Fra denne studien ble 0,02 mg furan/kg kroppsvekt/dag valgt som referansedose (point of departure, POD), basert på det nedre konfidensintervallet av benchmarkdosen ($BMDL_{10}$) på 0,14 mg furan/kg kroppsvekt/dag og en korreksjonsfaktor på 7 for kortere enn full livstids varighet (2 år) på studien.

Hovedkilden for furaneksponering hos 6, 12 og 24 måneder gamle barn er barnemat på glass. For 4, 9 og 13 år gamle barn er frokostblandinger den viktigste matkilde for furaneksponering. Hos voksne er det kaffe som gir det største bidraget til furaneksponering. Høyeste furaneksponering ble beregnet for 12 måneder gamle spedbarn og varierte fra 0,62 til

1,51 mikrogram/kg kroppsvekt/dag. Hos voksne varierte furaneksponeringen fra 0,27 til 0,82 mikrogram/kg kroppsvekt/dag.

Den gjennomsnittlig furan eksponering blant spedbarn, barn og ungdom ga MOE-verdier som varierte fra 29 hos 12 måneder gamle spedbarn til 2000 hos 13-årige ungdommer. Blant høykonsumenter i alle aldersgrupper varierte MOE-verdier fra 13 til 400. Hos voksne varierte MOE-verdiene fra 59 til 74 ved gjennomsnittlig furaneksponering, og fra 24 til 26 ved høy eksponering.

Det bemerkes at denne risikovurdering av furan inneholder vesentlige usikkerheter og begrensninger. Bruken av 9-måneders interrimstudie i rotte og en korreksjonsfaktor på 7 til å utlede en referansedose i stedet for en fullstendig livstidsstudie (2 år), overvurderer sannsynligvis faren ved furaneksponering. En mulig overdiagnostisering av kolangiokarsinom hos rotte på grunn av likheter i histopatologi mellom kolangiofibrose og kolangiokarsinom, kan også overvurdere faren. Det er begrensninger både i beregningene av matinntak i ulike aldersgrupper og av furaninnhold i ulike matvarer som igjen fører til usikkerhet i estimeringene av furaneksponeringene.

VKM anser at dagens eksponering for furan i alle aldersgrupper, særlig blant spedbarn og barn, er av bekymring for helsen.

Contents

Background

In 2004, the United States Food and Drug Administration (U.S. FDA) reported the presence of low furan concentrations in a wide variety of commonly consumed food, especially in foods that undergo thermal treatment such as canning and jarring. This discovery raised for the first time, concern about potential risk of furan to human health, even if furan had previously been identified in food such as coffee, canned food etc.

Knowledge on the toxicity of furan is rather limited, but at a meeting in February 2010 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that dietary exposure to furan resulted in hepatocellular adenomas and carcinomas in female mice (JECFA 2010, Summary report). The calculated Margin of Exposures (MOEs) representing average and high dietary human exposure to furan were considered by the Committee to indicate a human health concern. Furan is considered a possible carcinogen to humans (Group 2B) by the International Agency for Research on Cancer (IARC, 1995) and its carcinogenicity is probably attributable to a genotoxic mechanism (EFSA, 2004).

Risk assessments of furan have previously been performed by the European Food Safety Authority (EFSA) (2004) and by the Joint FAO/WHO Expert Committee on Food Additives (2011). Furan has also been evaluated by U.S. FDA (2004), and National Toxicology Program (NTP) (1993). Data on furan in food was reported by FDA (2004) and EFSA (2009; 2010; 2011), and in 2008 a small survey was performed by the Norwegian Food Safety Authority (Furan i næringsmidler, 2008). There are no Norwegian or European regulations that cover furan content in food.

Terms of reference

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety (VKM) to assess the significance of exposure to furan for the Norwegian population's health based on the concentrations found in food on the Norwegian market and on the most recent food consumption surveys for infants, small children, youths and adults (Spedkost, Småbarnskost, Ungkost, Norkost 3), and existing toxicological assessments of the substance.

- VKM is requested to assess the significance of exposure levels of furan for infants, children and youths based on data from Spedkost, Småbarnskost and Ungkost. The exposure assessment for adults should be based on data from the new food consumption survey Norkost 3.
- Where relevant, VKM should take into consideration occurrence data for concentrations of furan in foodstuffs from other countries in addition to the Norwegian data, where the foodstuffs in question (such as coffee) may contribute considerably to the exposure in the Norwegian population.

Assessment

1 Introduction

1.1 Previous risk assessments of furan

1.1.1 Initial Report of the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM) on provisional findings on furan in food (EFSA, 2004)

This report provided the current knowledge on exposure and adverse effects of furan and on gaps in the knowledge and possible research needs for future comprehensive risk assessments. The EFSA CONTAM Panel concluded, based on presently available data, that furan-induced carcinogenicity is probably attributable to a genotoxic mechanism, and that chronic toxicity with secondary cell proliferation may indirectly amplify the tumour response. There are limited sets of data on the occurrence of furan in various food categories, as well as available consumption data. Thus, EFSA chose to present the range of the estimated exposure rather than the average exposure. The exposure range of <0.03 – 3.5 µg/kg bw per day was based on furan concentrations from non-detectable to 112 µg/kg in baby food and an assumed consumption of 234 g/day of canned baby food of a 6 months old baby weighing 7.5 kg. However, the limited data revealed that there was relatively small difference between possible human exposure and the doses in experimental animals that produce carcinogenic effects. The EFSA CONTAM Panel also concluded that a reliable risk assessment would require further data on both toxicity and exposure.

1.1.2 72nd Joint FAO/WHO Expert Committee on Food Additives (2011) – Technical Report

At its 72nd meeting (2010), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a full evaluation of furan, as requested by the Codex Alimentarius Commission at its Thirty-first Session (2008). Results of studies on the genotoxic and carcinogenic potential of furan, as well as data on metabolism and disposition, short-term toxicity, reproductive developmental toxicity, perinatal carcinogenicity and immunotoxicity was assessed. In rats, but not mice, high incidences of cholangiocarcinomas were observed in all dose groups. Accordingly, no point of departure could be identified for this end point. Furthermore, the relevance of cholangiocarcinomas for humans was considered as unclear. Hepatocellular neoplasms were induced in both mice and rats, and a $BMDL_{10}$ of 1.3 mg/kg bw (corresponding to 0.96 mg/kg bw/day) for induction of hepatocellular adenomas and carcinomas in female mice was selected. The Committee suggested that the major route of exposure to furan is through consumption of heat-treated foods and beverages. A dietary exposure representing the average exposure of furan for the general population of 0.001 mg/kg bw/day and a dietary exposure of 0.002 mg/kg bw/day for consumers with high exposures was estimated. The highest estimate also covers the dietary exposure of children. Comparison of these exposure values with the selected $BMDL_{10}$, gives MOEs of 960 for average and 480 for high dietary exposures. It was considered by the Committee that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

1.2 Formation and persistence (or stability) of furan in foods

Figure 1: Furan, C_4H_4O

Furan (C_4H_4O, CAS-Nr. 110-00-9) is a volatile and lipophilic compound. In addition to its formation through thermal treatment of food, furan is also known as a by-product of high-energy radiation of food. Furthermore it serves as an intermediate in the synthesis and preparation of numerous polymers. The occurrence of furan in a variety of foods suggests that there are probably multiple routes of formation rather than a single mechanism. The proposed routes for furan formation are mainly based on Maillard reactions, thermal degradation of carbohydrates or certain amino acids, thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids, and free radical reactions during irradiation. The Danish Technical University has, in a project on behalf of EFSA, investigated the formation of furan in heat processed food products, including home-cooked foods. The results from this project indicated that foods with a high level of carbohydrates are most likely to produce furan formation. It was further shown that foods that were home-cooked using furan-containing ingredients did not lead to increased concentrations of furan in the prepared home-cooked foods (DTU, 2009). Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 ºC, 10 min) compared with pressure cooking conditions (sterilization, 121 ºC, 25 min), and pH plays a complex role in the mechanism of furan formation (JECFA, 2011).

Most results on furan concentrations in food items are derived from samples that were analysed as purchased, which means that only limited data are available on the formation of furan in home-cooked food as well as on furan stability during cooking, storage and reheating of meals (EFSA, 2010). Furan is stable in hot food and appears to be well dissolved within the food matrix. Despite its volatility, evaporation is believed to be hindered by slow diffusion inside the matrix (e.g. opening the jars of baby foods exposes only a relative small surface area). However, larger declines of furan content can be observed when canned and jarred foods are heated in a saucepan under stirring. In general, furan concentrations did not decrease as much when foods were heated in a microwave oven, as compared to the same food heated in a saucepan. Studies on reduction of furan concentration during warming procedures for ready-to-eat foods have shown conflicting results, with some authors reporting decreases of 29-85% in the furan concentration, and others finding persistent furan levels during normal heating practices. Decreases in furan concentrations in heated foods that are left for cooling, seem to be insignificant (JECFA, 2011; EFSA, 2010).

Furan evaporates to varying degrees from food during heating and may thus lead to exposure through inhalation of kitchen air. In 2009, EFSA published a scientific/technical report "Consumer exposure to furan from heat-processed foods and kitchen air", submitted by The Food and Environment Research Agency (FERA, 2009). In this project, the concentrations of furan were analysed in different types of foods before and after cooking, and also in the kitchen air during cooking. The results confirmed that furan exposure from diet depends on the food type and the cooking method. For cooking activities of short duration (<10 min),

furan concentrations in the air were low and variable, for cooking practices lasting more than 10 minutes data was more uniform. Concentrations up to about 10 ng/L of inhaled furan were calculated by multiplying the average furan content of the air in the cooking period (exposure period) with an assumed inhalation rate of 5 l/min. However, considerably more data on exhalation of furan is needed in order to calculate the net exposure from breathing. Accordingly, exposure by inhalation will not be further discussed in this opinion.

2 Furan contents in food

Furan is formed in a variety of heat-treated commercial foods and contributes to the sensory properties of the product. The substance has been found in a number of foods such as coffee, canned and jarred foods including baby food containing meat, and various vegetables. High concentrations of furan have been found in coffee and the presence of furan in jarred baby food and infant formulae has also received much attention since such products may form the sole diet for many infants.

2.1 Monitoring of furan contents in food reported by EFSA

In order to collect more information, EFSA issued a call for scientific data on furan in 2006 (EFSA, 2006a). In 2009, EFSA was asked by the European Commission to assemble occurrence data on furan in heat-treated commercial food products, collected by the Member states in 2007 and 2008 (EFSA, 2009). Reporting on occurrence data for furan now continues on a regular basis and a second EFSA report including data sampled and analysed between 2004 and 2009 was published in August 2010 (EFSA, 2010*)*. A new EFSA report with an update on furan levels in food from 2004-2010, including an exposure assessment was published in August 2011 (EFSA, 2011).

A total of 5050 analytical results for furan content in food, submitted by 20 countries between 2004 and 2010 have been reported and included in the latest update on furan levels from EFSA. The data have been sorted into 21 different food categories (5 coffee and 16 non-coffee categories) in accordance with previously reported results in the literature. The highest furan concentrations were found in coffee with mean values varying between 45 µg/kg for brewed coffee and 3660 µg/kg for roasted coffee beans. In the non-coffee categories, mean values ranged between 3.2 µg/kg for infant formula and 49 µg/kg for certain baby food categories (vegetables only). The highest concentrations (95^{th} percentile) reported were 6407 µg/kg for roasted coffee beans, and 123 µg/kg in jarred baby food (vegetables only) for the non-coffee categories (EFSA, 2011).

2.2 Norwegian data on furan contents in food

The Norwegian Food Safety Authority has in 2008 and 2009 collected data on furan concentrations in different food products sold in the Norwegian market (Norwegian Food Safety Authority, 2008). In all, 38 samples distributed into the following food categories; jarred baby food (24 samples), canned and jarred vegetables (4 samples), jarred sauces (8 samples) and baked beans (2 samples) were analysed. The data was collected in response to Commission Recommendation 2007/196/EC that requests Member States to monitor the presence of furan in foodstuffs that have undergone heat treatment and are included in the EFSA reports on furan levels in food published in 2010 and 2011 (EFSA, 2010; 2011).

In 2011, the Norwegian Food Safety Authority also decided to analyse commercial porridges for infants and children sold in the Norwegian market, to see if furan could be detected in

such products. A total of 14 commercial porridge samples was analysed; 12 powder-based porridges which should be mixed with water or milk and 2 ready-to-eat glass jars.

2.2.1 Sampling procedure and analytical method

All 38 Norwegian samples were analysed for the content of furan without any preparation of the purchased foodstuff. Thirty of the collected products were also analysed after further preparation as if consumed in the laboratory, e.g. canned and jarred products heated for consumption.

The sample preparation before analysis was carried out with care to ensure that the furan content of the samples was not altered. The furan concentrations in pooled samples of 3 identical products from the same producer were monitored. The products that were analysed after heat-treatment were heated in accordance with the instructions on the packaging.

The baby food samples (20 products) were transferred to a plate and microwave heated for 30 seconds at 750 watt. The pasta and dinner sauces (6 products) were transferred to a cup and gently boiled without a lid for 5 minutes. Canned vegetables (2 products) were heated with a similar procedure as the sauces. One of the 2 products of baked beans was heated after the liquid was removed and the beans were rinsed in water, while beans and liquid was homogenised and analysed together for the other product.

The 12 samples of powder-based commercial porridges were analysed for the furan content in the powder, without further preparation. The 2 ready-to-eat commercial porridges on glass jars were only analysed as purchased and not after heat-treatment.

The analytical method used for monitoring the furan concentrations was static headspace gas chromatography/mass spectrometry (GC-MS). All the food samples were analysed at the commercial laboratory Eurofins Analytic GmbH Wiertz-Eggert-Jörissen in Hamburg, Germany.

2.2.2 Reported LOD, LOQ and measurement uncertainty

The analytical method used was accredited in accordance with EN/ISO 17025 and validated for all relevant sample matrixes tested. The limit of detection (LOD) and limit of quantification (LOQ) reported for all matrixes tested were 2 and 5 µg/kg respectively. The measurement uncertainty was 80% for furan concentration below 20 µg/kg, while for concentrations higher than 20 µg/kg, the measurement uncertainty was 50%.

2.2.3 Results from the Norwegian monitoring of furan concentrations in food

The results from the analysis of the furan content in different food products sold in the Norwegian market in 2008, 2009 and 2011 are presented in Tables 1 to 5. The mean furan contents from the national monitoring data are reported as middle bound values, i.e. values below the LOQ (< 5 µg/kg) are set to the half of LOQ (2.5 µg/kg). This should be taken into consideration when the furan contents in different food samples are compared with occurrence data from the EFSA database, as the latter are reported as mean upper bound values (all values below LOQ are set to LOQ).

It should also be noted that considering the given uncertainties in the analyses (see section 2.2.2) the furan concentrations are presented with too many digits. However, VKM has chosen to use the furan concentrations as they were reported by the laboratory to ease tracing of the results used in this opinion back to the original data source.

2.2.3.1 Jarred baby food

An overview of the furan content in all jarred baby food samples analysed (both as purchased and after heat-treatment) in the Norwegian monitoring survey is shown in Table 1. In order to reflect the influence of ingredients on the furan content in jarred baby food, the results have been subdivided into different ingredient combinations in a similar way as in the monitoring reports of furan levels in food published by EFSA in 2010 and 2011 (EFSA, 2010; 2011). However, the different subcategories presented in Table 1 have also been adapted to which products that are included in the national food consumption surveys, i.e. the subcategory "fish and vegetables".

Table 1: Furan content in jarred baby food

Product (in Norwegian)	**Furan content (µg/kg)**					
	Analysed as purchased			**Analysed after heat-treatment**		
Meat and vegetables						
Nestlé Grønnsaker med kalkun (6 months)	24			22		
Nestlé Grønnsaker med lam (6 months)	39			28		
Småfolk Mors lapskaus (6 months)	29			29		
Småfolk Høstgryte med lam (6 months)	21			28		
Hipp Gulrøtter og mais med kalvekjøtt (6 months)	28			27		
Nestlé Favorittgryte kalkun (8 months)	60			48		
Småfolk Kjøttkaker (8 months)	49			51		
Småfolk Pasta bolognese (8 months)	25			42		
Hipp Grønnsak og kyllingrisotto (8 months)	56			63		
Nestlé Potet og kalkungryte (12 months)	46			45		
Nestlé Ris og kyllinggryte (12 months)	46			47		
Nestlé Spaghetti bolognese (12 months)	38			38		
Nestlé Lasagne (12 months)	52			114		
Småfolk Frikasé m/viltkjøtt (12 months)	32			35		
Nestlé Lapskaus (15 months)	36			27		
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Meat and vegetables (N=15)	**21**	**38.7**	**60**	**22**	**42.9**	**114**

Product (in Norwegian)	Furan content (µg/kg)					
	Analysed as purchased			Analysed after heat-treatment		
Vegetables						
Helios Gulrot med eple (4 months)	16			21		
Helios Blandede grønnsaker (6 months)	47			56		
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Vegetables (N=2)	**16**	**31.5**	**47**	**21**	**38.5**	**56**
Fruits						
Helios Pære/eple/havre (4 months)	<5			-		
Nestlé Frutti Eple Mango (4 months)	<5			-		
Nestlé Sviskemos (4 months)	20			-		
Nestlé Pære banan m/yoghurt (6 months)	7			-		
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Fruits (N=4)	**<5**	**8**	**20**	**-**	**-**	**-**
Cereal based						
Hipp Grønnsakslasagne (8 months)	50			50		
Hipp Pasta rigatoni (12 months)	65			51		
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Cereal based (pasta) (N=2)	**50**	**57.5**	**65**	**50**	**50.5**	**51**
Fish and vegetables						
Småfolk fiskeboller i hvit saus (8 months)	31			30		
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Fish and vegetables (N=1)	**31**	**31**	**31**	**30**	**30**	**30**
Jarred baby food (all products analysed independent of subcategories)						
	Min.	**Mean**	**Max.**	**Min.**	**Mean**	**Max.**
Jarred baby food (all products) (N=24)	**<5**	**34**	**65**	**21**	**43**	**114**

The results presented in Table 1 show that the furan content in jarred baby food containing meat and vegetables, only vegetables or cereals (pastas) were higher than in products containing fruits. This is in accordance with the results presented by EFSA in 2011 showing

that the mean upper bound furan content in the different baby food subcategories ranged from 5.3 µg/kg for baby food containing fruits only (N=250) to 49 µg/kg for baby food containing only vegetables (N=281). The mean furan content in Norwegian jarred baby food products containing meat and vegetables (38.7 µg/kg) was similar to the results reported by EFSA for this subcategory (40 µg/kg, N=550). For jarred cereal based (pasta) baby food sold on the Norwegian market, it was found a slightly higher furan content than what was reported by EFSA (57.5 versus 25 µg/kg), but this could be due to only 2 products being analysed in this subcategory. The baby food samples containing fish and vegetables had a mean furan content of 31 µg/kg.

The mean furan content in jarred baby food containing meat and vegetables which was analysed after heat-treatment was slightly higher than the same samples analysed as purchased. For cereal based (pasta) baby food, the opposite situation was found. It should be noted that only a limited number of samples was analysed in this survey, so the results should be interpreted with caution. None of the samples of jarred baby food containing fruits only were analysed after heat-treatment as such products are usually consumed cold.

Looking at the furan content in all the 24 samples of jarred baby food, not divided into different subcategories, concentrations in the range <5-65 µg/kg, with a mean value (middle bound) of 34 µg/kg, was found for products analysed as purchased. For the 20 samples which were also analysed after heat-treatment, the furan content varied from 21-114 µg/kg. The mean value of all the heat-treated samples was 43 µg/kg.

2.2.3.2 Commercial porridge

The furan content in 14 commercial porridges for infants and small children sold on the Norwegian market in 2011 is shown in Table 2. The 12 powder-based porridges analysed have been divided into two subcategories; 8 products with fruits and 4 products without fruits. Two products of ready-to-eat porridges on glass jars were also analysed.

Table 2: Furan content in commercial porridges

Products (in Norwegian)	Furan content (µg/kg)
Powder-based porridges with fruits	
Nestlé usukret musligrøt med pære og banan	38
Nestlé usukret havregrøt med pære og banan	28
Nestlé usukret fullkornsgrøt med frukt	32
Nestlé usukret havregrøt med eple	24
Småfolk mild fruktgrøt med eple og banan	<5
Småfolk havregrøt med banan, bringebær og yoghurt	<5
Småfolk mild fullkornsgrøt med pære, eple og bringebær	<5
Hipp mild grøt med frukt	<5
Mean value	16.5
Minimum – Maximum value	<5 - 38

Products (in Norwegian)	Furan content (µg/kg)
Powder-based porridges without fruits	
Nestlé usukret hvetegrøt med havre	<5
Småfolk kveldsgrøt med ris og pasta	<5
Hipp mild fullkornsgrøt	<5
Hipp fullkornsgrøt multikorn	<5
Mean value	2.5
Minimum – Maximum value	<5 - <5
Ready-to-eat porridges on glass jars	
Småfolk mild risgrøt med mango, banan og pære	<5
Småfolk havregrøt med eple og pære	<5
Mean value	2.5
Minimum – Maximum value	<5 - <5

The results presented in Table 5 show that 4 of all the commercial porridges analysed had furan contents above the LOQ. The mean middle bound furan content in the powder-based porridges with fruits was found to be 16.5 µg/kg. None of the powder-based porridges without fruits and the ready-to-eat porridges in glass jars had furan contents above LOQ. The mean furan content (middle bound) in these two subcategories of commercial porridges was 2.5 µg/kg.

Powder-based porridges with fruits were expected to have a higher furan content than powder-based porridges without fruits as furan have been found in several samples of dried fruits and vegetables (DTU, 2009).

2.2.3.3 Canned and jarred vegetables

The furan content in the four analysed products with canned and jarred vegetables is shown in Table 3.

Table 3: Furan content in canned and jarred vegetables

Manufacturer	Product (in Norwegian)	Furan content (µg/kg)	
		Analysed as purchased	Analysed after heat-treatment
Eldorado	Grovhakkede tomater, hermetiske	<5	<5
Eldorado	Hele/skrelte tomater, hermetiske	6	<5
Euroshopper	Sylteagurk	<5	-
Euroshopper	Rødbeter	<5	-

Mean value	**3.4**	**2.5**
Minimum – Maximum value	**<5-6**	**<5**

The mean furan content (middle bound) in canned and jarred vegetables, was found to be 3.4 µg/kg, with the highest value being 6 µg/kg in canned tomatoes. The results are in accordance with the results presented by EFSA in 2011 where the mean furan content in vegetables were 6.9-9.6 µg/kg (N=192). The 2 products with pickled vegetables were not analysed after heat-treatment, as they are usually consumed cold.

2.2.3.4 Jarred tomato sauces

Table 4 shows the furan content in the eight different jarred tomato sauces analysed.

Table 4: Furan content in jarred tomato sauces

Manufacturer	**Product (in Norwegian)**	**Furan content (µg/kg)**	
		Analysed as purchased	**Analysed after heat-treatment**
Dolmio	Classico (tomatsaus til pasta)	7	<5
Dolmio	Pastasaus ekstra hvitløk	10	5
Uncle Bens	Sweet and sour	<5	<5
Uncle Bens	Korma sauce	8	6
Barilla	Basilico Tomato-sauce with basilico for pasta	12	7
Sopps	Tomato-sauce for pasta	16	9
Santa Maria	Taco saus, Medium	11	-
Old El Paso	Taco saus, Medium	7	-
Mean value		**9.2**	**5.3**
Minimum – Maximum value		**<5-16**	**<5-9**

The furan content in the eight jarred tomato sauces analysed as purchased ranged from <5 to 16 µg/kg, with a mean furan content (middle bound) of 9.2 µg/kg. Slightly lower furan concentrations were found in the samples analysed after heat-treatment of the same products, with a mean middle bound furan content of 5.3 µg/kg. The results are in the same order of magnitude as the results presented for sauces by EFSA in 2011 where the mean furan content was 8.3-11 µg/kg (N=271). The two products with taco sauce were not analysed after heat-treatment.

2.2.3.5 Baked beans

The furan content in the two analysed products with baked beans is shown in Table 5.

Table 5: Furan content in baked beans

Manufacturer	Product (in Norwegian)	Furan content (µg/kg)	
		Analysed as purchased	Analysed after heat-treatment
Eldorado	Salat og grytebønner, hermetiske	6	<5
Coop	Tomatbønner	20	13
Mean value		**13**	**7.8**
Minimum – Maximum value		**6-20**	**<5-13**

The mean furan content in the two products with baked beans analysed as purchased was 13 µg/kg. Slightly lower concentrations were found for the samples that were analysed after heat-treatment, with a mean furan content (middle bound) of 9 µg/kg. The results for baked beans are somewhat lower compared with the results presented by EFSA in 2011 where a mean furan content of 22-24 µg/kg was found (N=57). However, it should be noted that the Norwegian results are based on analyses of only two products.

3 Exposure characterisation

3.1 Dietary exposure to furan

3.1.1 Norwegian data on furan contents in food used in the exposure assessment

The furan contents measured in various food products sold in the Norwegian market in 2008 and 2009 has been analysed both as purchased and after heat-treatment (Table 1-4). The furan content in powder based and ready-to-eat commercial porridges was only analysed as purchased (Table 2). For the exposure assessment in this opinion, VKM has chosen to use only the furan concentrations measured in products analysed as purchased, based on the Norwegian monitoring data. This decision is based on the high measurement uncertainty described in section 2.2.2 and the fact that the furan contents in identical food products analysed as purchased and after heat-treatment are of the same order of magnitude, except for a few products.

The calculated furan exposures in the exposure characterisation are based on the mean middle bound value of furan (analysed as purchased) found in the different food categories included in the Norwegian monitoring data (see section 2.2.3).

The two food categories canned and jarred vegetables and baked beans have been merged in the exposure calculations to simplify the linkage between the occurrence and food consumption data. The mean middle bound furan content in the 6 products analysed was calculated to 6.6 µg/kg (not shown), using the half of LOQ (2.5 µg/kg) for the three samples below LOQ. Consumption data on canned mackerel fillet in tomato sauce have been included in the exposure calculations for the food category jarred sauces, even though the Norwegian monitoring data only include occurrence data on jarred dinner sauces (see section 2.2.3.3).

The questionnaires used in the dietary surveys are not able to divide between commercial porridge with or without fruits. VKM has chosen a conservative approach in this risk assessment, where it is assumed that all commercial porridge consumed is porridge with fruits. Porridge with fruits has been found to have more than three times the amount of furan

compared with porridge without fruit. There is no data on ready-to-eat commercial porridges in the national food consumption surveys; therefore these products have not been included in the exposure calculations.

3.1.2 International data on furan contents in food used in the exposure assessment

National data on all relevant furan sources from food and beveragess is not available. For the exposure calculations of furan from other relevant sources than described in section 2.2, VKM has used the EFSA database on occurrence data on furan in heat-treated commercial food products (EFSA, 2011). If considered relevant, occurrence data on furan reported in the scientific literature or in research projects has also been used (DTU, 2009). It should be noted that the mean upper bound furan values reported in the EFSA database have been used in the exposure calculations, whereas middle bound furan values have been used for the national monitoring data and for the data from the research project carried out at the Danish Technical University National Food Institute.

VKM is aware of a comprehensive set of analytical data on furan contents in various food samples reported in the scientific literature and from other governmental agencies (e.g. Kuballa 2007; Zoller *et al*., 2007; Becalski *et al*., 2010; FSA, 2012; Lachenmeier *et al*., 2012). However, for the exposure characterisation in this VKM opinion, it was decided to emphasize the national monitoring data on furan and the occurrence data available in the EFSA furan database.

A description of the food categories and respective furan concentrations that have been included in the exposure calculations in this opinion from VKM is given in the following sections.

3.1.2.1 Infant formula

No occurrence data for furan content in infant formula sold in the Norwegian market is available. The calculated furan exposures have therefore been based on the mean furan content (upper bound) in dry infant formulae of 3.2 µg/kg (N=11), as reported in the most recent EFSA monitoring report (EFSA, 2011). A conversion factor of 0.119 was applied to the food consumption data, as they were reported for liquid infant formula (prepared), to convert them back to dry infant formulae and to map the consumption with the occurrence data for furan from the EFSA report (EFSA, 2009; 2010).

3.1.2.2 Canned fruits

No data on the furan content is available for products sold in the Norwegian market. The mean furan content (upper bound) of 6.4 µg/kg (N=142), reported for the product category fruits in the most recent monitoring report from EFSA (EFSA, 2011), has been used in the exposure calculations.

3.1.2.3 Milk products

Since no furan concentration data for milk products sold in the Norwegian market is available, the concentration data reported by EFSA was used in the exposure calculations. The mean furan content (upper bound) of 5.6 µg/kg (N=64) reported for milk based processed food in the most recent monitoring report from EFSA (EFSA, 2011) has been linked to Norwegian consumption data for milk products (yoghurts, cheeses). It should, however, be noted that there is no detailed information on which foods have been included in the food category milk products in the EFSA report. Milk as such was not included as a relevant furan

source, as all milk samples present in the database on existing data for furan in food from the EU-project FURAN-RA (SSPE-CT-2006-44393) contained furan levels below the LOQ (VUA, 2007).

3.1.2.4 Chocolate and sweets

The mean furan content (upper bound) of 6 µg/kg (N=61) for the food category sweets in the EFSA report from 2011 has been used to calculate the furan exposure based on the national consumption data for chocolate and sweets in the exposure calculations.

3.1.2.5 Coffee

Since there is no data available on the furan content in Norwegian coffee, the occurrence data for coffee brew, subcategorised according to different coffee types presented in the EFSA report from 2011 are used in the exposure calculations. From the EFSA data, it could be observed that the mean furan content was considerably lower for coffee brew samples than for the respective solid coffee samples (see Table 5 and 8 in EFSA, 2011). The mean furan contents (upper bound) in coffee instant brew of 7 µg/L (N=15) and in coffee, roasted ground, brew of 42 µg/L (N=51) have been linked with the consumption data for coffee in the national consumption surveys.

3.1.2.6 Breakfast cereals

Results from a project carried out by the Danish Technical University National Food Institute on behalf of EFSA showed high contents of furan in breakfast cereals, products highly consumed by children. A total of 11 breakfast cereal products, including 2 honey-coated products were analysed in the Danish project. The furan concentrations ranged from <2.4 to a high value of 387 µg/kg in one of the honey-coated products (DTU, 2009). The national food consumption data include information on both sweet breakfast cereals (Honny korn, Frosties, Chocofrokost and Ricekrisp) and ordinary breakfast cereals. To link the national food consumption data to the Danish occurrence data, VKM has used the mean middle bound furan contents in the 2 honey-coated breakfast cereals of 224 µg/kg (N=2) and in the ordinary breakfast cereals of 20.7 µg/kg (N=9) in the exposure calculations.

These furan values reported in the Danish project were chosen rather than the upper bound value of 18 µg/kg (N=190) reported for cereal products in the EFSA furan database (EFSA, 2011), since the breakfast cereal products in the Norwegian market are assumed to be more similar to the products in the Danish market, than in the other EU countries.

3.1.2.7 Snacks and crisps

Snacks and crisps are other food products analysed in the Danish furan project. A total of 9 products were analysed and the furan concentrations varied from <2.4 - 90.6 µg/kg, of which the highest concentration was found in popcorn. The mean middle bound furan content in the Danish investigation was calculated to be 24.7 µg/kg. This is somewhat higher, compared to the furan data for snacks and crisps presented by EFSA in 2011, where a mean furan content (upper bound) of 10 µg/kg was reported (N=133). It was decided to use the mean furan content from the Danish furan project (DTU, 2009) in the exposure calculations since snacks and crisps sold in the Danish and Norwegian markets are considered to be more similar than all products included for this product category in the EFSA furan database.

3.1.2.8 Overview of the mean furan contents in the food categories included in the exposure calculations

An overview of the mean furan contents used in the exposure calculations for the various food categories included in this opinion is presented in Table 6.

Table 6: Mean furan content in the various food categories included in the exposure calculations.

Food category	N	Furan content (µg/kg)	Source
Jarred baby food (meat and vegetables)	15	38.7[a]	National monitoring data (analysed as purchased)
Jarred baby food (vegetables)	2	31.5[a]	National monitoring data (analysed as purchased)
Jarred baby food (fruits)	4	8[a]	National monitoring data (analysed as purchased)
Jarred baby food (cereal based (pasta))	2	57.5[a]	National monitoring data (analysed as purchased)
Jarred baby food (fish and vegetables)	1	31[a]	National monitoring data (analysed as purchased)
Canned and jarred vegetables (including baked beans)	6	6.6[a]	National monitoring data (analysed as purchased)
Jarred tomato sauces	8	9.2[a]	National monitoring data (analysed as purchased)
Commercial porridges (powder based containing fruits)	8	16.5[a]	National monitoring data (analysed as purchased in powder)
Commercial porridges (powder based without fruits)	4	2.5[a]	National monitoring data (analysed as purchased in powder)
Infant formula	11	3.2[b]	EFSA database on furan levels (2011)
Canned fruits	142	6.4[b]	EFSA database on furan levels (2011)
Milk products (yoghurts, cheeses)	64	5.6[b]	EFSA database on furan levels (2011)
Chocolate and sweets	61	6[b]	EFSA database on furan levels (2011)
Coffee, roasted ground, brew	51	42[b]	EFSA database on furan levels (2011)
Coffee, instant, brew	15	7[b]	EFSA database on furan levels (2011)
Sweet breakfast cereals	2	224[c]	DTU report (2009)
Breakfast cereals	9	20.7[c]	DTU report (2009)
Snacks and crisps	9	24.7[c]	DTU report (2009)

[a] Mean middle bound values from the Norwegian monitoring data.
[b] Mean upper bound values from the EFSA furan database (EFSA, 2011).
[c] Mean middle bound values from the Danish furan report (DTU, 2009).

3.1.3 Methodological description of the national consumption surveys

In the present opinion, the calculated furan exposures from food and beverages are based on data from the nationally representative food consumption surveys; Spedkost, Småbarnskost, Ungkost and Norkost. The consumption for each relevant food and food category in the dietary surveys were multiplied with the corresponding mean furan concentrations as described in section 3.1.1 and 3.1.2 and totalled for each individual.

A short description of the consumption surveys and the different methodologies used is given below:

- **6-month-old infants;** Spedkost 2006-2007 is based on a semi-quantitative frequency questionnaire. The study was conducted in 2006, and a total of 1986 6-month-old infants participated (Øverby *et al.*, 2008).
- **1-year-old infants;** Spedkost 2006-2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1635 1-year-old infants participated (Øverby *et al*., 2009).
- **2-year-old children;** Småbarnskost 2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1674 2-year-olds participated (Kristiansen *et al*., 2009).
- **4-year-old children;** Ungkost 2000 is based on a 4-day food intake registration with a precoded food diary. Food amounts were presented in predefined household units or as portions estimated from photographs (Pollestad *et al*., 2002). The study was conducted in 2001, and 391 4-year-olds participated.
- **9- and 13-year-old children/adolescents;** Ungkost 2000 is based on a 4-day food intake registration with a precoded food diary. Food amounts were presented in predefined household units or as portions estimated from photographs (Øverby and Andersen, 2002). The study was conducted in 2000 and 810 9-year-old children and 1005 13-year-old adolescents participated.
- **Adults;** Norkost 3 is based on two 24-hour recalls by telephone at least one month apart. Food amounts were presented in household measures or estimated from photographs (Totland *et al*., 2012). The study was conducted in 2010/2011 and 1787 adults aged 18-70 years participated.

Daily consumption of foods containing furan was computed by using food databases in the software system (KBS) developed at the Institute of Basic Medical Sciences, Department of Nutrition, at the University of Oslo. The food databases are mainly based on various versions of the official Norwegian Food Composition Table (Rimestad *et al*., 2000, The Norwegian Food Composition Table 1995 and 2006). The three dietary surveys that are the basis for this risk assessment were conducted at three different time points, Ungkost-2000 in 2000-2001, Sped- and Småbarnskost in 2007 and the data for Norkost 3 were collected in 2010-2011. The national furan analyses were conducted in the time period 2008-2011.

3.1.3.1 Breastfed and non-breastfed infants

The exposure assessment for furan in the Norwegian population has been performed on two different groups in the dietary surveys for infants 6 and 12 months of age. The furan exposure has been calculated for all participants, both breastfed and non-breastfed, and for the group of infants that were not breastfed. The calculation was performed to see if the two groups varied

in consumption of furan-containing food products and if that could lead to higher exposure estimates in one of the groups.

3.1.3.2 Consumers only

The tables describing the consumption of food categories in each age group are based on persons who actually reported a consumption of the relevant food (consumers only) in the dietary survey.

Furthermore, the risk assessment has one table for each age group where the furan exposure for the consumers of the most important furan source in that age group is presented. The furan exposure is calculated in consumers only of this most important age specific furan source, with an additional contribution from all relevant food categories.

3.1.3.3 Body weight

The mean body weights reported for the different age groups in the dietary surveys have been used to calculate the exposure in mg furan/kg bw. An overview of these body weights is given in Table 7.

Table 7: Mean body weight of different age groups in the Norwegian population.

Age	Mean body weight (kg)
6-month-old infants	8
12-month-old infants	9.9
24-month-old children	12.8
4-year-old children	18
9-year-old children	32
13-year-old adolescents	49.5
Adults aged 18-70 years	77.5

3.1.4 Dietary exposure to furan in 6-month-old infants (Spedkost 2006)

3.1.4.1 Jarred baby food

An overview of the consumption of different subcategories jarred baby food and exposure to furan in 6-month-old infants is given in Table 8. The mean and 95 percentile consumptions of jarred baby food are calculated based on the frequency of consumption reported in the dietary survey and the amount of baby food assumed to be consumed per meal (1/4 jar ≈ 50 g) (Øverby *et al*., 2008).

The furan exposures have been calculated based on the mean furan contents in the different subcategories of jarred baby food analysed as purchased (see Table 1 and 6) and on the mean and 95 percentile consumptions shown in Table 8. A calculation of the furan exposure from the total of jarred baby food consumed has also been included, based on the individual consumption data reported for 6-month-old infants. All values in Table 8 are given for consumers only among all participants (n=1986) in the dietary survey.

Table 8: Consumption of different subcategories jarred baby food and exposure to furan in 6-month-old infants (n=1986). Values for consumers only within each subcategory are given.

		Frequency of consumption, times/day		Amount consumed per meal, g	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Baby food subcategory	**Number of consumers (% of 1986)**	**Mean (SD)**	**95 percentile**	**1/4 jar of baby food**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Meat and vegetables	578 (29)	0.7 (0.4)	1.0	50	37 (19)	50	0.18	0.24
Vegetables	479 (24)	0.6 (0.4)	1.0	50	30 (18)	50	0.12	0.20
Fish and vegetables	107 (5)	0.5 (0.3)	1.0	50	26 (16)	50	0.10	0.19
Fruits[b]	1028 (52)	0.9 (0.7)	2.0	50	45 (33)	100	0.05	0.10
Total jarred baby food	1220 (61)	1.4 (0.9)	3.0	50	70 (47)	150	**0.18**	**0.45**

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Include berries but not juice.

The results show that jarred baby food containing meat and vegetables gave the highest contribution with mean and 95 percentile furan exposures of 0.18 and 0.24 µg/kg bw/day, respectively. The mean furan exposure from the total of jarred baby food consumed by 6-month-old infants was 0.18 µg/kg bw/day, while the exposure at the 95 percentile was found to be 0.45 µg/kg bw/day.

Similar calculations have been carried out for consumers only of non-breastfed participants in the dietary survey (n=397). The mean and 95 percentile furan exposures from consumption of the total of jarred baby food in this group of 6-month-old infants was found to be 0.23 and 0.49 µg/kg bw/day, respectively (see Table A1 in Appendix I).

3.1.4.2 Infant formula

The furan exposure from consumption of infant formula in 6-month-old infants is shown in Table 9. The furan exposure has been calculated based on the mean furan content in dry infant formula reported in the EFSA database (see Table 6) and on the mean and 95 percentile consumption of infant formula reported for all participants and for the non-breastfed infants in the dietary survey (consumers only for both groups).

The results presented in Table 9 show a mean furan exposure from consumption of infant formula of 0.02 µg/kg bw/day for consumers only among all participants. The exposure at the 95 percentile was 0.05 µg/kg bw/day. For the group of infants that were not breastfed, respective mean and 95 percentile furan exposures of 0.03 and 0.06 µg/kg bw/day were calculated.

Table 9: Furan exposure from dry infant formula in 6-month-old infants (n=1986) and among non-breastfed participants (n=397). Values are given for consumers only.

Infant formula	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a,b]	
		Mean (SD)	95 percentile	Mean	95 percentile
Consumers only among all participants (n=1986)	728 (37)	61 (40)[c]	114[c]	0.02	0.05
Consumers only among non-breastfed participants (n=397)	388 (98)	84 (31)[c]	143[c]	0.03	0.06

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Based on a mean furan content in dry infant formula of 3.2 µg/kg (EFSA, 2011).
[c] Conversion factor from wet to dry infant formula of 0.119 was used (EFSA, 2009).

Table 9 shows values for dry infant formula. Converted to liquid volume the consumption of 61 g of dry infant formula equals 513 g of ready-to-drink infant formula.

3.1.4.3 Commercial powder-based porridge

The furan exposure from consumption of powder-based commercial porridge in 6-month-old infants is shown in Table 10. The furan exposure has been calculated based on the mean furan contents in porridge powder with fruits and without fruits (see Table 2) and on the mean and 95 percentile consumption of porridge reported for all participants and for the non-breastfed infants in the dietary survey (consumers only for both groups).

Table 10: Furan exposure from commercial powder-based porridge in 6-month-old infants (n=1986) and non-breastfed participants (n=397). Values are given for consumers only. Furan exposure is shown in two scenarios, where all porridge powder is assumed either with fruits (16.5 µg furan/kg) or without fruits (2.5 µg furan/kg).

Type of commercial porridge	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Consumers only among all participants (n=1986)					
Porridge powder with fruits[b]	1652 (83)	32 (31)	89	0.07	0.18
Porridge powder without fruits[b]	1652 (83)	32 (31)	89	0.01	0.03
Consumers only among non-breastfed participants (n=397)					
Porridge powder with fruits[b]	369 (93)	44 (36)	104	0.09	0.21
Porridge powder without fruits[b]	369 (93)	44 (36)	104	0.01	0.03

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Amount porridge powder derived from standardised recipes in the KBS software.

The results in Table 10 show that the respective mean and 95 percentile furan exposures from consumption of commercial powder-based porridge with fruits were 0.07 and 0.18 µg/kg bw/day for consumers only among all participants. Mean and 95 percentile furan exposures of 0.09 and 0.21 µg/kg bw/day were calculated for the group of infants that was not breastfed.

Consumption of powder-based commercial porridges without fruits resulted in mean and 95 percentile furan exposures of 0.01 and 0.03 µg/kg bw/day for consumers only of all participants. In the group of infants that were not breastfed, mean and 95 percentile furan exposures from consumption of porridges without fruits were 0.01 and 0.03 µg/kg bw/day, respectively.

Table 10 shows values for dry porridge powder. To get a picture of how much powder is needed to get the mean ready-to-eat volume of porridge among all participants in the dietary survey; 32 g of porridge powder equals 163 g of ready-to-eat porridge.

3.1.4.4 Furan exposure in 6-month-old infants

Three different estimates for the furan exposure in Norwegian 6-month-old infants are shown in Table 11 (all participants) and 12 (non-breastfed participants). First an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of jarred baby food only (also presented in Table 8 and Table A1). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of jarred baby food with an additional contribution from infant formula and commercial powder-based porridge with fruits was calculated (see Table 9 and 10). Finally, an estimate that shows the average furan exposure from jarred baby food, infant formula and commercial powder-based porridge with fruits for all participants (Table 11) and for all non-breastfed participants (Table 12) in the dietary survey, was calculated.

Table 11: Furan exposure in 6-month-old infants for consumers of jarred baby food (n=1220), for consumers of jarred baby food with an additional contribution from infant formula and commercial powder-based porridge (n=1220) and average furan exposure in all participants (n=1986).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b] (SD)	95 percentile, µg/kg bw/day[a,b]
Consumers of jarred baby food[c]	1220 (61)	0.18 (0.14)	0.45
Consumers of jarred baby food with an additional contribution from infant formula and commercial powder-based porridge	1220 (61)	0.26 (0.18)	0.58
Average furan exposure from jarred baby food, infant formula and commercial powder-based porridge in all participants	1986 (100)	0.17 (0.18)	0.51

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Based on a mean furan content in jarred baby food, infant formula and porridge powder with fruits as reported in Table 6.
[c] Exposure estimate presented in Table 8.

The results in Table 11 show that 6-month-old consumers of jarred baby food have mean and 95 percentile furan exposures of 0.18 and 0.45 µg/kg bw/day, respectively. Taking into account the additional contribution from infant formula and commercial powder-based

porridge with fruits, the mean and 95 percentile furan exposures increase to respective 0.26 and 0.58 µg/kg bw/day. In the third estimate showing the average furan exposure from jarred baby food, infant formula and commercial powder-based porridge with fruits for all participants in the dietary survey, respective mean and 95 percentile values of 0.17 and 0.51 µg/kg bw/day were found.

Table 12: Furan exposure in 6-month-old non-breastfed infants for consumers of jarred baby food (n=331), for consumers of jarred baby food with an additional contribution from infant formula and commercial powder-based porridge (n=331) and average furan exposure in all non-breastfed participants (n=397).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b] (SD)	95 percentile, µg/kg bw/day[a,b]
Consumers of jarred baby food[c]	331 (83)	0.23 (0.14)	0.49
Consumers of jarred baby food with an additional contribution from infant formula and commercial powder-based porridge	331 (83)	0.35 (0.17)	0.65
Average furan exposure from jarred baby food, infant formula and commercial powder-based porridge in all non-breastfed participants	397 (100)	0.31 (0.19)	0.62

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Based on a mean furan content in jarred baby food, infant formula and porridge powder with fruits as reported in Table 6.
[c] Exposure estimate presented in Table A1.

Table 12 shows that the mean and 95 percentile furan exposures in the group of 6-month-old non-breastfed consumers of jarred baby food were calculated to 0.23 and 0.49 µg/kg bw/day, respectively. In the estimate which includes an additional contribution from infant formula and commercial powder-based porridge with fruits, the mean and 95 percentile furan exposures increase to 0.35 and 0.65 µg/kg bw/day. The average furan exposures from jarred baby food, infant formula and commercial powder-based porridge with fruits in all non-breastfed participants resulted in mean and 95 percentile values of 0.31 and 0.62 µg/kg bw/day, respectively.

The relative contribution from various food categories to the furan exposure in 6-month-old infants is illustrated in Figure 2 (all participants, n=1986) and 3 (all non-breastfed participants, n=397).

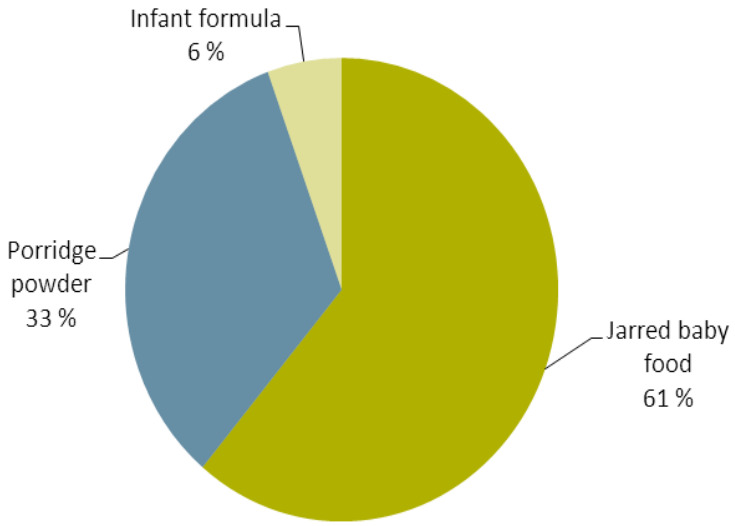

Figure 2: The relative contribution from various food categories (jarred baby food, porridge powder and infant formula) to furan exposure in 6-month-old infants (all participants, n=1986).

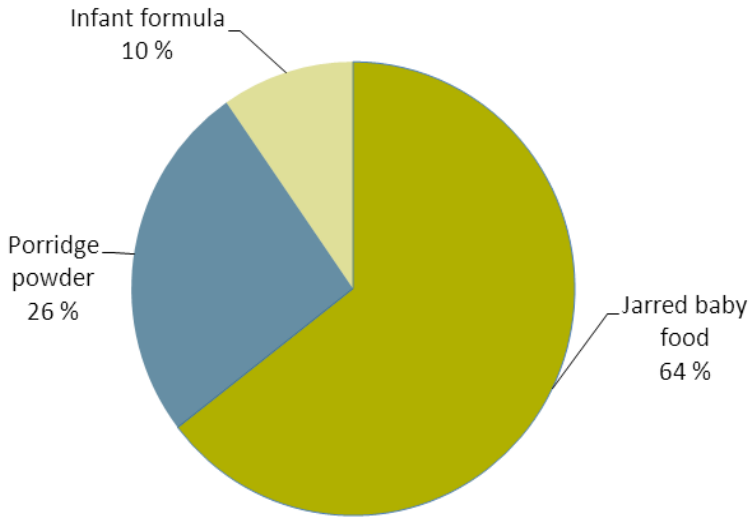

Figure 3: The relative contribution from various food categories (jarred baby food, porridge powder and infant formula) to furan exposure in 6-month-old infants (all non-breastfed participants, n=397).

Consumption of jarred baby food resulted in the largest relative contribution to the furan exposure in 6-month-old infants, both among all participants in the dietary survey (61%) and in the group of non-breastfed participants (64%). The relative contribution from porridge powder was larger in the group of all participants (33%) compared to the non-breastfed participants (26%), while the relative contribution from infant formula was largest in non-breastfed infants (10%).

3.1.5 Dietary exposure to furan in 12-month-old infants (Spedkost 2007)

3.1.5.1 Jarred baby food

An overview of the consumption of different subcategories jarred baby food and exposure to furan in 12-month-old infants is shown in Table 13. The furan exposures have been calculated based on the mean furan contents in jarred baby food analysed as purchased (see Table 1 and 6) and on the mean and 95 percentile consumption of the different subcategories of jarred baby food reported in the dietary survey. A calculation of the furan exposure from the total of jarred baby food consumed has also been included, based on the individual consumption data reported for 12-month-old infants. All values in Table 13 are given for consumers only among all participants (n=1986) in the dietary survey.

Table 13: Consumption of different subcategories jarred baby food and exposure to furan in 12-month-old infants (n=1635). Values for consumers only within each subcategory are given.

		Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Baby food subcategory	**Number of consumers (% of 1635)**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Meat and vegetables	1185 (72)	112 (82)	249	0.44	0.97
Fish and vegetables	585 (36)	54 (31)	139	0.17	0.43
Cereal based	265 (16)	53 (33)	139	0.31	0.80
Vegetables	144 (9)	55 (39)	183	0.17	0.58
Fruits[b]	1157 (71)	83 (78)	226	0.07	0.18
Total jarred baby food	1436 (88)	196 (155)	447	**0.56**	**1.30**

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Includes berries but not juice.

The calculations presented in Table 13 show that jarred baby food containing meat and vegetables gave the highest contribution to the furan exposure with mean and 95 percentile values of 0.44 and 0.97 µg/kg bw/day, respectively. The mean furan exposure from the total of jarred baby food consumed by this age group was 0.56 µg/kg bw/day, while the exposure at the 95 percentile was 1.30 µg/kg bw/day.

Similar calculations have been performed for consumers only of non-breastfed participants in the dietary survey (n=881). The furan exposures from consumption of the total of jarred baby food in this group of infants were in the same order of magnitude as what was calculated for all participants, with mean and 95 percentile furan exposures of 0.58 and 1.33 µg/kg bw/day, respectively (see Table A2 in Appendix I).

3.1.5.2 Infant formula

The furan exposure from consumption of infant formula, in 12-month-old infants, is shown in Table 14. The furan exposure has been calculated based on the mean upper bound furan content in dry infant formula reported in the EFSA database (see Table 6) and on the mean and 95 percentile consumption of infant formula reported for all participants and for the non-breastfed infants in the dietary survey (consumers only for both groups).

Table 14: Furan exposure from dry infant formula in 12-month-old infants (n=1635) and among non-breastfed participants (n=881). Values are given for consumers only.

Infant formula	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a,b]	
		Mean (SD)	95 percentile	Mean	95 percentile
Consumers only among all participants (n=1635)	654 (40)	43 (27)[c]	86[c]	0.01	0.03
Consumers only among non-breastfed participants (n=881)	516 (59)	47 (27)[c]	86[c]	0.02	0.03

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Based on a mean furan content in infant formula of 3.2 µg/kg (EFSA, 2011).
[c] Conversion factor from wet to dry infant formula of 0.119 was used (EFSA, 2009).

The calculations resulted in a mean furan exposure from consumption of infant formula of 0.01 µg/kg bw/day for consumers only among all participants. The exposure at the 95 percentile was 0.03 µg/kg bw/day. The group of infants that were not breastfed had mean and 95 percentile furan exposures of 0.02 and 0.03 µg/kg bw/day, respectively.

Table 14 shows values for dry infant formula. Converted to liquid volume the consumption of 43 g of dry infant formula equals 360 g of ready-to-drink infant formula.

3.1.5.3 Milk products

The furan exposure from consumption of milk products (yoghurt, cheese) in 12-month-old infants is presented in Table 15. Milk as such has not been included as a relevant furan source (VUA, 2007). The furan exposure has been calculated based on the mean upper bound furan content in milk products reported in the EFSA database (see Table 6) and on the mean and 95 percentile consumption of milk products (yoghurt, cheese) reported for all participants and for the non-breastfed infants in the dietary survey (consumers only for both groups).

Table 15: Furan exposure from milk products (yoghurt, cheese) in 12-month-old infants (n=1635) and among non-breastfed participants (n=881). Values are given for consumers only.

Milk products (yoghurt, cheese)	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a,b]	
		Mean (SD)	95 percentile	Mean	95 percentile
Consumers only among all participants (n=1635)	1494 (91)	57 (56)	154	0.03	0.09
Consumers only among non-breastfed participants (n=881)	821 (93)	61 (55)	161	0.03	0.09

Consumption of milk products (yoghurt, cheese) was reported for up to 93% of 12-month-old infants. The mean furan exposure was 0.03 µg/kg bw/day both for consumers only among all participants and in the group of participants that were not breastfed. The 95 percentile furan exposures also showed similar results for the two groups of participants, with an exposure of 0.09 µg/kg bw/day.

3.1.5.4 Commercial powder-based porridge

The furan exposure from consumption of commercial powder-based porridge in 12-month-old infants is shown in Table 16. The furan exposure has been calculated based on the mean furan contents in porridge powder with fruits and without fruits (see Table 2) and on the mean and 95 percentile consumption of commercial porridge reported for all participants and for the non-breastfed infants in the dietary survey (consumers only for both groups).

Table 16: Furan exposure from commercial powder-based porridge in 12-month-old infants (n=1635) and non-breastfed participants (n=881). Values are given for consumers only. Furan exposure is shown in two scenarios, where all porridge is assumed either with fruits (16.5 µg furan/kg) or without fruits (2.5 µg furan/kg).

		Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Type of commercial porridge	**Number of consumers (%)**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Consumers only among all participants (n=1635)					
Porridge powder with fruits[b]	1348 (82)	69 (51)	166	0.12	0.28
Porridge powder without fruits[b]	1348 (82)	69 (51)	166	0.02	0.04
Consumers only among non-breastfed participants (n=881)					
Porridge powder with fruits[b]	713 (81)	66 (46)	140	0.11	0.23
Porridge powder without fruits[b]	713 (81)	66 (46)	140	0.02	0.04

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Amount porridge powder derived from standardised recipes in the KBS software.

The calculations show mean and 95 percentile furan exposures from consumption of commercial powder-based porridge with fruits of 0.12 and 0.28 µg/kg bw/day, respectively, for consumers only among all participants. In the group of consumers that were not breastfed, mean and 95 percentile furan exposures of 0.11 and 0.23 µg/kg bw/day were found.

Consumption of powder-based commercial porridges without fruits resulted in mean and 95 percentile furan exposures of 0.02 and 0.04 µg/kg bw/day for consumers only among all participants. The respective values in the group of infants that were not breastfed were 0.02 and 0.04 µg/kg bw/day.

Table 16 shows values for dry porridge powder. To get a picture of how much powder is needed to get the mean ready-to-eat volume of porridge among all participants in the dietary survey; 69 g of porridge powder equals 321 g of ready-to-eat porridge.

3.1.5.5 Furan exposure in 12-month-old infants

Three different estimates for the furan exposure in Norwegian 12-month-old infants are shown in Table 17 (all participants) and 19 (non-breastfed participants). First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of jarred baby food (also presented in Table 13 and Table A2). This food category was found to be the most important furan source in this age group. Secondly, an

estimate for the consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge with fruits was calculated (see Tables 14, 15 and 16). The third estimate shows the average furan exposure from jarred baby food, infant formula, milk products and commercial powder-based porridge with fruits for all participants (Table 17) and for all non-breastfed participants (Table 18) in the dietary survey.

Table 17: Furan exposure in 12-month-old infants for consumers of jarred baby food (n=1436), for consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge (n=1436) and average furan exposure in all participants (n=1635).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of jarred baby food[c]	1436 (88)	0.56	1.30
Consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge	1436 (88)	0.69	1.51
Average furan exposure from jarred baby food, infant formula, milk products and commercial powder-based porridge in all participants	1635 (100)	0.62	1.43

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Based on a mean furan content in jarred baby food, infant formula, milk products and porridge powder with fruits as reported in Table 6.
[c] Exposure estimate presented in Table 13.

The results in Table 17 show that the mean and 95 percentile furan exposures in 12-month-old consumers of jarred baby food were 0.56 and 1.30 µg/kg bw/day, respectively. Looking at the additional contributions from infant formula, milk products and commercial powder-based porridge with fruits, the mean and 95 percentile exposure values increase to 0.69 and 1.51 µg/kg bw/day. In the third estimate illustrating the average furan exposure from all food categories considered to be relevant for all participants in this age group, mean and 95 percentile values of 0.62 and 1.43 µg/kg bw/day were calculated.

Table 18: Furan exposure in 12-month-old non-breastfed infants for consumers of jarred baby food (n=777), for consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge (n=777) and average furan exposure in all non-breastfed participants (n=881).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of jarred baby food[c]	777 (88)	0.58	1.33
Consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge	777 (88)	0.72	1.54
Average furan exposure from jarred baby food, infant formula, milk products and commercial powder-based porridge in all non-breastfed participants	881 (100)	0.65	1.47

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Based on a mean furan content in jarred baby food, infant formula, milk products and porridge powder with fruits as reported in Table 6.
[c] Exposure estimate presented in Table A2.

Table 18 shows that consumers of jarred baby food in the group of 12-month-old non-breastfed infants have respective mean and 95-percentile furan exposures of 0.58 and 1.33 µg/kg bw/day. In the estimate that includes an additional contribution from infant formula, milk products and commercial powder-based porridge with fruits, the mean and 95 percentile furan exposures increase to 0.72 and 1.54 µg/kg bw/day. The calculation of the average furan exposures from jarred baby food, infant formula, milk products and commercial powder-based porridge with fruits in all non-breastfed participants resulted in mean and 95 percentile values of 0.65 and 1.47 µg/kg bw/day, respectively.

The relative contribution from various food categories to the furan exposure in 12-month-old infants is illustrated in Figure 4 (all participants, n=1635).

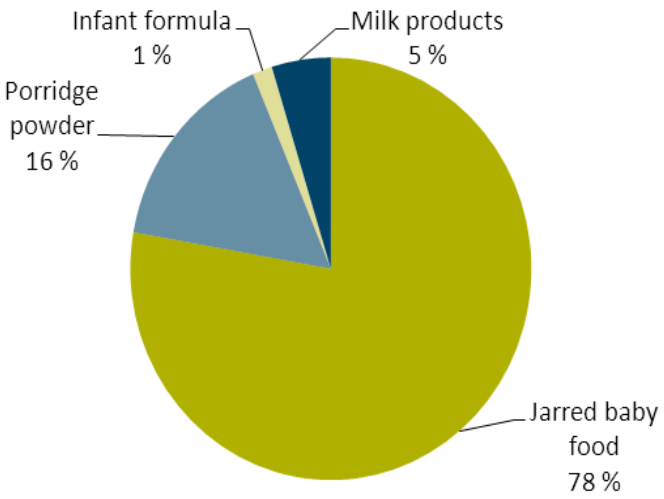

Figure 4: The relative contribution from various food categories (jarred baby food, porridge powder, infant formula and milk products) to furan exposure in 12-month-old infants (all participants, n=1635).

Consumption of jarred baby food represented the largest relative contribution to the furan exposure in 12-month-old infants (78%). The relative contribution from porridge powder amounted to 16%, while consumption of milk products gave a relative contribution of 5%. Consumption of infant formula is less important in 12-month-old infants with a relative contribution of only 1%.

The relative contribution from various food categories in 12-month-old non-breastfed infants gave almost identical results and is therefore not shown.

3.1.6 Dietary exposure to furan in 24-month-old children (Småbarnskost 2007)

3.1.6.1 Jarred baby food

The consumption of different subcategories jarred baby food with the corresponding furan exposure from this food category in 24-month-old children is presented in Table 19. The furan exposures have been calculated based on the mean furan contents in jarred baby food analysed as purchased given in Tables 1 and 6 and on the mean and 95 percentile consumption of the different subcategories of jarred baby food reported in the dietary survey. A calculation of the furan exposure from the total of jarred baby food consumed has also been included, based on the individual consumption data reported for 24-month-old children. All

values in Table 19 are given for consumers only among all participants (n=1674) in the dietary survey.

Table 19: Consumption of different subcategories jarred baby food and exposure to furan in 24-month-old children (n=1674). Values for consumers only within each subcategory are given.

		Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Baby food subcategory	**Number of consumers (% of 1674)**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Meat and vegetables	198 (12)	87 (53)	195	0.26	0.59
Fish and vegetables	68 (4)	54 (29)	139	0.13	0.34
Cereal based	35 (2)	49 (21)	73	0.22	0.33
Vegetables	19 (1)	54 (44)	195	0.13	0.48
Fruits[b]	275 (16)	34 (49)	148	0.02	0.09
Total jarred baby food	420 (25)	79 (93)	258	**0.18**	**0.64**

[a] Calculated based on the mean body weight of 12.8 kg reported for 24-month-old children (see Table 7).
[b] Include berries but not juice.

From Table 19, it can be seen that jarred baby food containing meat and vegetables resulted in the highest furan exposures with respective mean and 95 percentile values of 0.26 and 0.59 µg/kg bw/day. The mean furan exposure from the total of jarred baby food consumed by 24-month-old children was calculated to 0.18 µg/kg bw/day, while the exposure at the 95 percentile was 0.64 µg/kg bw/day.

3.1.6.2 Other relevant food categories

The consumption of other food categories considered relevant for 24-month-old children, with the corresponding furan exposures, is shown in Table 20. The furan exposure have been calculated based on the furan contents given for relevant food categories in Table 6 and on the mean and 95 percentile consumption of these food categories reported in the dietary survey. The following food categories have been included for this age group: canned and jarred vegetables, canned fruits, milk products (yoghurt, cheese), jarred tomato sauces, sweet breakfast cereals, breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge with and without fruits. All results were calculated based on individual consumption data from the participants in the dietary survey (consumers only).

Table 20: Consumption of relevant food categories and exposure to furan in 24-month-old children (n=1674). Values are given for consumers only.

		Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Food category	**Number of consumers (% of 1674)**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Canned and jarred vegetables	1298 (77)	0 (0)	1	<0.01	<0.01
Canned fruits	50 (3)	17 (12)	58	<0.01	0.03

Food category	Number of consumers (% of 1674)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Milk products (yoghurt, cheese)	1492 (89)	79 (64)	205	0.03	0.09
Jarred tomato sauces	1446 (86)	2 (3)	7	<0.01	<0.01
Sweet breakfast cereals	147 (9)	2 (1)	5	0.03	0.09
Breakfast cereals[b]	819 (49)	11 (11)	33	0.02	0.05
Chocolate, sweets	1293 (77)	4 (3)	10	<0.01	<0.01
Snacks	1079 (64)	1 (1)	4	<0.01	<0.01
Commercial powder-based porridge with fruits	285 (17)	31 (31)	89	0.04	0.11
Commercial powder-based porridge without fruits	285 (17)	31 (31)	89	<0.01	0.02

[a] Calculated based on the mean body weight of 12.8 kg reported for 24-month-old children (see Table 7).
[b] Ordinary breakfast cereals excluding sweet breakfast cereals.

Table 20 shows that consumption of commercial powder-based porridge with fruits resulted in the highest furan exposure from other food categories than jarred baby food in 24-month-old children. The mean and 95 percentile furan exposures from consumption of this food category were calculated to 0.04 and 0.11 µg/kg bw/day, respectively. Consumption of milk products and sweet breakfast cereals resulted in respective mean and 95 percentile furan exposures of 0.03 and 0.09 µg/kg bw/day. The furan exposures from consumption of other relevant food categories in this age group were found to be of limited importance.

3.1.6.3 Furan exposure in 24-month-old children

Three different estimates for the furan exposure in Norwegian 24-month-old children are shown in Table 21. First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of jarred baby food (also presented in Table 19). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of jarred baby food with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks and commercial powder-based porridge was calculated (see Table 20). Finally, an estimate showing the average furan exposure from all food categories considered relevant for this age group for all participants in the dietary survey was calculated.

Table 21: Furan exposure in 24-month-old children for consumers of jarred baby food (n=420), for consumers of jarred baby food with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge (n=420) and average furan exposure in all participants (n=1674).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of jarred baby food[c]	420 (25)	0.18	0.64
Consumers of jarred baby food with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge	420 (25)	0.25	0.77
Average furan exposure from jarred baby food, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge in all participants	1674 (100)	0.10	0.41

[a] Calculated based on the mean body weight of 12.8 kg reported for 24-month-old children (see Table 7).
[b] Based on the furan contents in jarred baby food, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge with fruits as reported in Table 6.
[c] Exposure estimate presented in Table 19.

Table 21 shows respective mean and 95 percentile furan exposures in 24-month-old consumers of jarred baby food of 0.18 and 0.64 µg/kg bw/day. Taking into account the additional contribution from other food categories considered relevant for this age group, the mean and 95 percentile furan exposures for consumers of jarred baby food increased to 0.25 and 0.77 µg/kg bw/day. The calculation of the average furan exposure from all relevant food categories in all participants resulted in a mean value of 0.10 µg/kg bw/day and a 95 percentile value of 0.41 µg/kg bw/day.

3.1.7 Dietary exposure to furan in 4-year-old children (Ungkost 2001)

3.1.7.1 Relevant food categories

The consumption of different food categories considered relevant for 4-year-old children, with the corresponding furan exposures, is presented in Table 22. The furan exposures have been calculated based on the furan contents given for relevant food categories in Table 6 and on the mean and 95 percentile consumption of these food categories reported in the dietary survey. The following food categories have been included for this age group: canned and jarred vegetables, canned fruits, milk products (yoghurt, cheese), jarred tomato sauces, sweet breakfast cereals, breakfast cereals, chocolate and sweets, and snacks. All results were calculated based on individual consumption data from the participants in the dietary survey (consumers only).

Table 22: Consumption of relevant food categories and exposure to furan in 4-year-old children (n=391). Values are given for consumers only.

Food category	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Canned and jarred vegetables	102 (26)	11 (10)	28	<0.01	0.01
Canned fruits	12 (3)	33 (25)	88	0.01	0.03
Milk products (yoghurt, cheese)	352 (90)	36 (45)	143	0.01	0.04
Jarred tomato sauces	150 (38)	23 (23)	61	0.01	0.03
Sweet breakfast cereals	65 (17)	14 (11)	34	0.18	0.43
Breakfast cereals[b]	182 (47)	22 (30)	95	0.03	0.11
Chocolate, sweets	349 (89)	14 (13)	43	<0.01	0.01
Snacks	168 (43)	6 (7)	25	<0.01	0.03

[a] Calculated based on the mean body weight of 18 kg reported for 4-year-old children (see Table 7).
[b] Ordinary breakfast cereals excluding sweet breakfast cereals.

The calculations presented in Table 22 show that consumption of breakfast cereals (especially sweet breakfast cereals) gave the highest contribution to furan exposure from relevant food categories in 4-year-old children. Respective mean and 95 percentile furan exposures of 0.03 and 0.11 µg/kg bw/day were found for breakfast cereals, while consumption of sweet breakfast cereals resulted in mean and 95 percentile exposures of 0.18 and 0.43 µg/kg bw/day. Since both the number of consumers with a reported consumption in the dietary survey (see Table 22) and the number of products analysed (DTU, 2009) were higher for breakfast cereals than sweet breakfast cereals, the food category breakfast cereals was considered the most important furan source in this age group. The furan exposures from consumption of the other food categories included in the exposure calculations shown in Table 22 were found to be low.

3.1.7.2 Furan exposure in 4-year-old children

Three different estimates for furan exposure in Norwegian 4-year-old children are shown in Table 23. First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of breakfast cereals (also presented in Table 22). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks was calculated (see Table 22). The third estimate shows the average furan exposure from all relevant food categories considered relevant for this age group among all participants in the dietary survey.

Table 23: Furan exposure in 4-year-old children for consumers of breakfast cereals (n=182), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=182) and average furan exposure in all participants (n=391).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of breakfast cereals[c]	182 (47)	0.03	0.11
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks	182 (47)	0.08	0.26
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks in all participants	391 (100)	0.07	0.25

[a] Calculated based on the mean body weight of 18 kg reported for 4-year-old children (see Table 7).
[b] Based on the furan contents in relevant food categories reported in Table 6.
[c] Exposure estimate presented in Table 22.

From Table 23 it can be seen that the additional contribution from other relevant food categories would increase the mean and 95 percentile furan exposures for the consumers of breakfast cereals from 0.03 and 0.11 µg/kg bw/day (exposure from breakfast cereals) to 0.08 and 0.26 µg/kg bw/day, respectively. The average furan exposure from all relevant food categories considered relevant for all participants in the dietary survey resulted in mean and 95 percentile furan exposures of 0.07 and 0.25 µg/kg bw/day, respectively.

3.1.8 Dietary exposure to furan in 9-year-old children (Ungkost 2000)

3.1.8.1 Relevant food categories

The consumption of different food categories considered relevant for 9-year-old children, with the corresponding furan exposures, is presented in Table 24. The furan exposures have been calculated based on the furan contents given for relevant food categories in Table 6 and on the mean and 95 percentile consumption of these food categories reported in the dietary survey. The following food categories have been included for this age group: canned and jarred vegetables, canned fruits, milk products (yoghurt, cheese), jarred tomato sauces, sweet breakfast cereals, breakfast cereals, chocolate and sweets, and snacks. All results were calculated based on individual consumption data from the participants in the dietary survey (consumers only).

Table 24: Consumption of relevant food categories and exposure to furan in 9-year-old children (n=810). Values are given for consumers only.

Food category	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Canned and jarred vegetables	237 (29)	13 (11)	37	<0.01	<0.01
Canned fruits	62 (8)	26 (30)	88	<0.01	0.02
Milk products (yoghurt, cheese)	731 (90)	73 (75)	208	0.01	0.04
Jarred tomato sauces	274 (34)	35 (40)	123	0.01	0.04
Sweet breakfast cereals	125 (15)	21 (23)	75	0.15	0.52
Breakfast cereals[b]	323 (40)	34 (44)	136	0.02	0.09
Chocolate, sweets	727 (90)	31 (29)	84	<0.01	0.02
Snacks	459 (57)	14 (21)	50	0.01	0.04

[a] Calculated based on the mean body weight of 32 kg reported for 9-year-old children (see Table 7).
[b] Ordinary breakfast cereals excluding sweet breakfast cereals.

The results presented in Table 24 show that consumption of breakfast cereals (especially sweet breakfast cereals) gave the highest contribution to furan exposure from the food categories considered relevant for 9-year-old children. The mean furan exposure from breakfast cereals was calculated to 0.02 µg/kg bw/day, while the exposure at the 95 percentile was 0.09 µg/kg bw/day. Consumption of sweet breakfast cereals could result in mean and 95 percentile furan exposures of 0.15 and 0.52 µg/kg bw/day, respectively. Since both the number of consumers with a reported consumption in the dietary survey (see Table 24) and the number of products analysed (DTU, 2009) were higher for breakfast cereals than sweet breakfast cereals, the food category breakfast cereals was considered the most important furan source in this age group. Consumption of milk products (yoghurt, cheese) and snacks could both lead to a furan exposure of 0.04 µg/kg bw/day at the 95 percentile, but overall the furan exposure from other food categories than breakfast cereals was found to be low in 9-year-old children.

3.1.8.2 Furan exposure in 9-year-old children

Three different estimates for furan exposure in Norwegian 9-year-old children are shown in Table 25. First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of breakfast cereals (also presented in Table 24). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks was calculated (see Table 24). Finally, an estimate showing the average furan exposure from all food categories considered relevant for this age group among all participants in the dietary survey was calculated.

Table 25: Furan exposure in 9-year-old children for consumers of breakfast cereals (n=323), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=323) and average furan exposure in all participants (n=810).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of breakfast cereals[c]	323 (40)	0.02	0.09
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks	323 (40)	0.06	0.17
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks in all participants	810 (100)	0.06	0.20

[a] Calculated based on the mean body weight of 32 kg reported for 9-year-old children (see Table 7).
[b] Based on the mean furan content in relevant food categories (Table 6).
[c] Exposure estimate presented in Table 24.

The exposure calculations presented in Table 25 show that the mean furan exposure for 9-year-old consumers of breakfast cereals would increase from 0.02 (exposure from breakfast cereals) to 0.06 µg/kg bw/day when the additional contributions from other relevant food categories were taken into account. The furan exposure at the 95 percentile for the consumers of breakfast cereals was found to be 0.17 µg/kg bw/day when all relevant food sources are included. The average furan exposure from all relevant food categories considered relevant for all participants in the dietary survey resulted in a mean and 95 percentile furan exposures of 0.06 and 0.20 µg/kg bw/day, respectively.

3.1.9 Dietary exposure to furan in 13-year-old adolescents (Ungkost 2000)

3.1.9.1 Relevant food categories

The consumption of different food categories considered relevant for 13-year-old adolescents, with the corresponding furan exposures, is presented in Table 26. The furan exposures have been calculated based on the furan contents given for relevant food categories in Table 6 and on the mean and 95 percentile consumption of these food categories reported in the dietary survey. The following food categories have been included for this age group: canned and jarred vegetables, canned fruits, milk products (yoghurt, cheese), jarred tomato sauces, sweet breakfast cereals, breakfast cereals, chocolate and sweets, snacks and coffee (coffee roasted ground). All results were calculated based on individual consumption data from the participants in the dietary survey (consumers only).

Table 26: Consumption of relevant food categories and exposure to furan in 13-year-old adolescents (n=1005). Values are given for consumers only.

Food category	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Canned and jarred vegetables	351 (35)	19 (19)	52	<0.01	<0.01
Canned fruits	73 (7)	27 (34)	100	<0.01	0.01
Milk products (yoghurt, cheese)	875 (87)	74 (86)	224	<0.01	0.03
Jarred tomato sauces	248 (25)	38 (49)	105	<0.01	0.02
Sweet breakfast cereals	116 (12)	24 (24)	70	0.11	0.32
Breakfast cereals[b]	285 (28)	35 (43)	112	0.01	0.05
Chocolate, sweets	911 (91)	44 (45)	130	<0.01	0.02
Snacks	594 (59)	26 (40)	88	0.01	0.04
Coffee roasted ground	13 (1)	55 (21)	100	0.05	0.08

[a] Calculated based on the mean body weight of 49.5 kg reported for 13-year-old adolescents (see Table 7).
[b] Ordinary breakfast cereals excluding sweet breakfast cereals.

As for 4- and 9-year-old children, consumption of sweet breakfast cereals resulted in the highest contribution to furan from the food categories considered relevant for 13-year-old adolescents (Table 26). The mean furan exposure from sweet breakfast cereals was calculated to 0.11 µg/kg bw/day, while the exposure at the 95 percentile was 0.32 µg/kg bw/day. Consumers of ordinary breakfast cereals were found to have mean and 95 percentile furan exposures of 0.01 and 0.05 µg/kg bw/day. Since both the number of consumers with a reported consumption in the dietary survey (see Table 26) and the number of products analysed (DTU, 2009) were higher for breakfast cereals than sweet breakfast cereals, the food category breakfast cereals was considered the most important furan source in this age group. It should be noted that mean and 95 percentile furan exposures of 0.05 and 0.08 µg/kg bw/day were calculated for consumption of coffee roasted ground. However, due to the low number of consumers (N=13), coffee was not considered as an important furan source in 13-year-old adolescents. Consumption of milk products and snacks could lead to respective furan exposures of 0.03 and 0.04 µg/kg bw/day at the 95 percentile, but overall the furan exposure to other food categories was found to be low in this age group.

3.1.9.2 Furan exposure in 13-year-old adolescents

Three different estimates for furan exposure in Norwegian 13-year-old adolescents are shown in Table 27. First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of breakfast cereals (also presented in Table 26). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, snacks, and coffee (see Table 26). The third estimate

shows the average exposure from all relevant food categories considered relevant for this age group among all participants in the dietary survey.

Table 27: Furan exposure in 13-year-old adolescents for consumers of breakfast cereals (n=285), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=285) and average furan exposure in all participants (n=1005).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of breakfast cereals[c]	285 (28)	0.01	0.05
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, snacks, and coffee	285 (28)	0.05	0.16
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, snacks, and coffee in all participants	1005 (100)	0.04	0.14

[a] Calculated based on the mean body weight of 49.5 kg reported for 13-year-old adolescents (see Table 7).
[b] Based on the mean furan content in relevant food categories (Table 6).
[c] Exposure estimate presented in Table 26.

From Table 27 it can be seen that the additional contribution from other relevant food categories would increase the mean and 95 percentile furan exposures for the consumers of breakfast cereals from 0.01 and 0.05 µg/kg bw/day (exposure from breakfast cereals) to 0.05 and 0.16 µg/kg bw/day, respectively. The average exposure from all relevant food categories considered relevant for all participants in the dietary survey resulted in mean and 95 percentile furan exposures of 0.04 and 0.14 µg/kg bw/day, respectively.

3.1.10 Dietary exposure to furan in adults aged 18-70 years (Norkost 3)

3.1.10.1 Relevant food categories

The consumption of different food categories considered relevant for adults aged 18-70 years, with the corresponding furan exposures, is presented in Table 26. The furan exposures have been calculated based on the furan contents given for relevant food categories in Table 6 and on the mean and 95 percentile consumption of these food categories reported in the dietary survey. The following food categories have been included for adults: canned and jarred vegetables, canned fruits, milk products (yoghurt, cheese), jarred tomato sauces, sweet breakfast cereals, breakfast cereals, chocolate and sweets, snacks and coffee (coffee roasted ground and instant coffee). All results were calculated based on individual consumption data from the participants in the dietary survey (consumers only).

Table 28: Consumption of relevant food categories and exposure to furan in adults aged 18-70 years (n=1787). Values are given for consumers only.

Food category	Number of consumers (%)	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
		Mean (SD)	95 percentile	Mean	95 percentile
Canned and jarred vegetables	274 (15)	23 (34)	91	<0.01	<0.01
Canned fruits	121 (7)	29 (35)	87	<0.01	<0.01
Milk products (yoghurt, cheese)	1605 (90)	74 (70)	204	<0.01	0.01
Jarred tomato sauces	478 (27)	30 (32)	100	<0.01	0.01
Sweet breakfast cereals	7 (0)	30 (6)	-	0.09	-
Breakfast cereals[b]	491 (27)	60 (54)	174	0.02	0.05
Chocolate, sweets	1229 (69)	18 (23)	64	<0.01	<0.01
Snacks	277 (16)	31 (33)	75	<0.01	0.02
Coffee roasted ground	1398 (78)	604 (456)	1500	0.33	0.81
Instant coffee	266 (15)	319 (303)	997	0.03	0.09

[a] Calculated based on the mean body weight of 77.5 kg reported for adults aged 18-70 years (see Table 7).
[b] Ordinary breakfast cereals excluding sweet breakfast cereals.

The calculations presented in Table 28 show that consumption of coffee gave the highest contribution to furan exposure from relevant food categories in adults aged 18-70 years. Consumption of coffee roasted ground resulted in mean and 95 percentile furan exposures of 0.33 and 0.81 µg/kg bw/day, respectively, while consumption of instant coffee gave respective mean and 95 percentile values of 0.03 and 0.09 µg/kg bw/day. Consumption of breakfast cereals could result in mean and 95 percentile furan exposures of 0.02 and 0.05 µg/kg bw/day, but overall the furan exposures from other relevant food categories, except coffee, were found to be low in the exposure calculations for adults.

3.1.10.2 Furan exposure in adults aged 18-70 years

Three different estimates for furan exposure in Norwegian adults aged 18-70 years are shown in Table 29. First, an estimate based on individual consumption data for the participants in the dietary survey with a reported consumption of coffee roasted ground (also presented in Table 28). This food category was found to be the most important furan source in this age group. Secondly, an estimate for the consumers of coffee roasted ground with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee (see Table 28). The third estimate shows the average exposure from all relevant food categories considered relevant for this age group among all participants in the dietary survey.

Table 29: Furan exposure in adults for consumers of coffee roasted ground (n=1398), for consumers of coffee roasted ground with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee (n=1398) and average furan exposure in all participants (n=1787).

	Number of consumers (%)	Mean, µg/kg bw/day[a,b]	95 percentile, µg/kg bw/day[a,b]
Consumers of coffee roasted ground[c]	1398 (78)	0.33	0.81
Consumers of coffee roasted ground with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee	1398 (78)	0.34	0.82
Average furan exposure from coffee roasted ground, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee in all participants	1787 (100)	0.27	0.77

[a] Calculated based on the mean body weight of 77.5 kg reported for adults aged 18-70 years (see Table 7).
[b] Based on the mean furan content in relevant food categories (Table 6).
[c] Exposure estimate presented in Table 28.

Table 29 shows respective mean and 95 percentile furan exposures in adults with a consumption of coffee roasted ground of 0.33 and 0.81 µg/kg bw/day. The mean and 95 percentile furan exposures for consumers of coffee roasted ground increased to 0.34 and 0.82 µg/kg bw/day, respectively, when the additional contribution from other relevant food categories in this age group was taken into account. The calculation of the average furan exposure from all relevant food categories in all participants resulted in a mean value of 0.27 µg/kg bw/day and a 95 percentile value of 0.77 µg/kg bw/day, respectively.

The relative contribution from various food categories to the furan exposure in adults aged 18-70 years is illustrated in Figure 5 (all participants, n=1787).

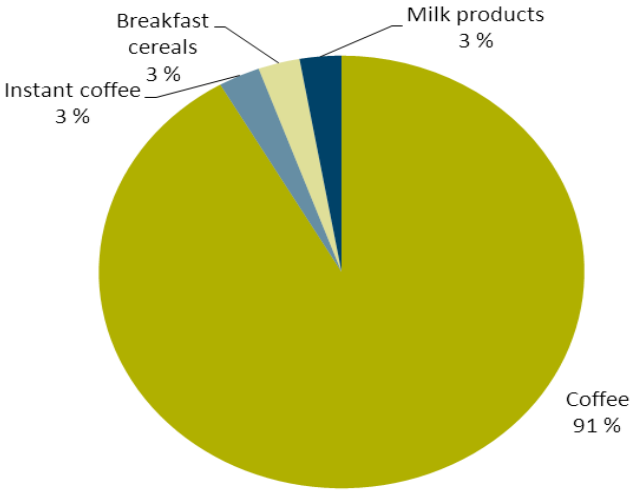

Figure 5: The relative contribution from four food categories (coffee, instant coffee, breakfast cereals, milk products) to furan exposure in adults in all participants (n=1787).

Consumption of coffee (coffee roasted ground) resulted in the largest relative contribution to the furan exposure in adults aged 18-70 years, amounting to as much as 91% of the overall exposure. Consumption of instant coffee, breakfast cereals and milk products all gave a relative contribution of 3%.

3.1.11 Summary of exposure assessments for furan in the Norwegian population

An overview of the calculated furan exposures from various food categories considered relevant for the different age groups is shown in Table 30 and 31. Table 30 shows the population average of furan exposure among all participants in each age group, and also the average among all non-breastfed 6- and 12-month-old children. Furthermore, the 95-percentiles of furan exposure among all participants in each age group are shown. In Table 31, the furan exposures for the consumers of the most important furan source (values given for consumers only) with an additional contribution from all relevant food categories are presented for each age group.

Table 30: Furan exposure from food and beverages for all participants in different age groups in Norway (population average)

Age groups	Number of consumers (%)	Mean, µg/kg bw/day[a]	95 percentile, µg/kg bw/day[a]
6 months old children	1986 (100)	0.17	0.51
6 months old children (non-breastfed)	397 (100)	0.31	0.62
12 months old children	1635 (100)	0.62	1.43
12 months old children (non-breastfed)	881 (100)	0.65	1.47
24 months old children	1674 (100)	0.10	0.41
4 years old children	391 (100)	0.07	0.25
9 years old children	810 (100)	0.06	0.20
13 years old children	1005 (100)	0.04	0.14
Adults	1787 (100)	0.27	0.77

[a] Calculated based on the mean body weights for different age groups reported in the dietary surveys (see Table 7).

Table 31: Furan exposure from food and beverages for consumers of the most important furan sources in different age groups in Norway (values given for consumers only).

Age groups	Number of consumers (%)	Mean, µg/kg bw/day[a]	95 percentile, µg/kg bw/day[a]
6 months old children	1220 (61)	0.26	0.58
6 months old children (non-breastfed)	331 (83)	0.35	0.65
12 months old children	1436 (88)	0.69	1.51
12 months old children (non-breastfed)	777 (88)	0.72	1.54
24 months old children	420 (25)	0.25	0.77
4 years old children	182 (47)	0.08	0.26

Age groups	Number of consumers (%)	Mean, µg/kg bw/day[a]	95 percentile, µg/kg bw/day[a]
9 years old children	323 (40)	0.06	0.17
13 years old children	285 (28)	0.05	0.16
Adults	1398 (78)	0.34	0.82

[a] Calculated based on the mean body weights for different age groups reported in the dietary surveys (see Table 7).

The highest population average was found in 12-month-old infants with a mean furan exposure of 0.62 µg/kg bw/day (0.65 µg/kg bw/day in non-breastfed infants). Adults also had a high population average with a mean furan exposure of 0.27 µg/kg bw/day, while 13-year-old adolescents was found to have the lowest mean furan exposure (0.04 µg/kg bw/day) based on the consumption of all relevant furan-containing food categories (Table 30).

If the furan exposures were calculated for the consumers of the most important food source with an additional contribution from all relevant food categories (Table 31), slightly higher or similar furan exposures as for the population averages were found in all age groups. This indicates that the population averages are a realistic exposure estimate for different consumers, also for those who consume foods with a high furan content.

3.1.12 Comparison with previous exposure assessments of furan

EFSA has in their third scientific report on the results of the monitoring of furan levels in Europe between 2004 and 2010 also presented furan exposure estimates based on the monitoring results (EFSA, 2011). The exposure to furan was estimated for different target populations by combining pooled mean furan occurrence values from all Member States obtained through the monitoring program with individual dietary national consumption data from Member States derived from the EFSA Comprehensive European Food Consumption Database.

The mean furan exposure across surveys was estimated to range between 0.03 and 0.59 µg/kg bw/day for adults, between 0.02 and 0.13 µg/kg bw/day for adolescents, between 0.04 and 0.22 µg/kg bw/day for other children, between 0.05 and 0.31 µg/kg bw/day for toddlers and between 0.09 and 0.22 µg/kg bw/day for infants. High exposure at the 95 percentile was found to be from 0.06 to 1.38 µg/kg bw/day in adults, while the surveys covering toddlers and other children had respective 95 percentiles ranged between 0.20 and 1.40 and 0.09 and 0.46 µg/kg bw/day. Only two surveys related to infants are included in the EFSA Comprehensive Database and a possible 95 percentile furan exposure of 0.97 µg/kg bw/day was reported (EFSA, 2011).

Brewed coffee was the main contributor to the exposure in adults, with an average of 85% of total furan exposure. Fruit juice, milk-based products and cereal-based products was important contributors to the furan exposure in toddlers and other children, whereas in addition for toddlers jarred baby food were found to be a major contributor (EFSA, 2011).

Estimates of dietary exposure to furan have also been included in the recent JECFA evaluation of furan (JECFA, 2011). In general, the mean dietary exposure to furan from national assessments ranged from 0.25 to 1.17 µg/kg bw/day for adults, from 0.08 to 0.23 µg/kg bw/day for children 1-6 years of age and from 0.27 to 1.01 µg/kg bw/day for infants up to 12 months of age. For consumers at high percentiles of dietary exposure, estimates ranged

from 0.60 to 2.22 µg/kg bw/day for adults and from 0.99 to 1.34 µg/kg bw/day for infants. No high-percentile data were reported for children. (JECFA, 2011).

A dietary exposure assessment based on Danish consumption data has been presented in the report from the project carried out by the Danish Technical University National Food Institute on behalf of EFSA (DTU, 2009). The result revealed that 95% of the furan exposure in adults came from consumption of coffee. For Danish children, breakfast cereals were found to be the food category contributing most to the furan exposure. For children with a high consumption of breakfast cereals (the 95 percentile) and a mean consumption of other foods the furan exposure from breakfast cereals accounted for about 2/3 of the total exposure. The estimate of the total median furan exposure for adults (15-75 years old) was calculated to 33.5 µg/day and for children (4-6 years old) to 1.1 µg/day (DTU, 2009).

4 Hazard identification and characterisation

4.1 Toxicokinetics

Furan is a small lipophilic molecule (MW: 68.07 g/mol) that easily can pass through biological membranes. Pharmacokinetic ADME parameters (absorption, distribution, metabolism, and excretion) of furan have been determined in a number of *in vitro* and *in vivo* studies (Egle and Gochberg, 1979; Burka *et al*., 1991; Wilson *et al*, 1992; Kedderis *et al*., 1993; Carfagna *et al*., 1993; Kedderis and Held, 1996; Chen *et al*., 1995; Peterson *et al*., 2005; Gill *et al*. 2010; Peterson *et al*., 2011). However, data are not complete and mostly based on rodent studies. Disposition characteristics of furan in humans are not available.

Compiled ADME information on furan can also be retrieved from EFSA (2004) and JECFA (2011) reports as well as from a publication of the US National Research Council's Subcommittee on Spacecraft Maximum Allowable Concentrations (2000).

4.1.1 Absorption

Furan was rapidly absorbed from the intestine into the portal vein after oral administration to rats and mice (Burka *et al*. 1991; Wilson *et al*., 1992). Portal vein blood flow is the limiting step for furan deliverance to the liver in all species investigated (Kedderis *et al*., 1993). However, oral application of high doses as used in toxicology studies (50 mg/kg bw) can cause direct diffusion through the stomach walls into the systemic circulation. In a study on absorption by inhalation 90 % of the dose was taken up in dogs and mice, and 95 % in rats (Egle and Gochberg, 1979). The amount of retained furan was proportional to the furan concentration in inhaled air. Physiological-based pharmacokinetic models have been used to develop dosimetry models for furan by inhalation showing that the absorbed dose in humans would be about 3 to 10-fold smaller than in rats and mice, respectively (Kedderis and Held, 1996). It could be speculated that the ratio would be similar after oral administration.

4.1.2 Distribution

A single oral dose of 8 mg/kg bw radiolabelled furan [2,5-^{14}C] in corn oil to rats resulted in recoveries of 307 nmol/g furan equivalents in the liver, 60 nmol/g in the kidneys, 44 nmol/g in the gastrointestinal tract, 6 nmol/g in blood, and 4 nmol/g in the lungs at 24 h post administration (p.a.) (Burka *et al*., 1991). The labelled equivalents represented about 15% of the total furan dose. They were almost completely bound to protein. Seven days p.a.,

radioactivity in the different organs was below the detection limit. Repeated dosing led to accumulation of radioactivity in liver and kidneys (Burka *et al*., 1991). In a 90-day gavage toxicity study in rats, unlabelled furan at doses 0.0, 0.03, 0.12, 0.5, 2.0, and 8.0 mg/kg bw/day five days a week were applied (Gill *et al*., 2010). After 90 days, furan residues in the liver for the sentinel, controls and animals dosed with 0.03 and 0.12 mg/kg bw/day were almost similar and ranging from 0.05 to 0.08 ng/g liver tissue. Animals dosed with 0.5, 2.0, and 8.0 mg/kg bw/day had about 0.11 ng/g, 0.21 ng/g and 0.30 ng/g furan in the liver tissue, respectively.

4.1.3 Metabolism

Furan is metabolized quickly by cytochrome P450 enzymes, mainly CYP 2E1, to form cis-2-butene-1,4-dial (BDA), which has been identified as the major primary metabolite (Burka *et al*., 1991; Adger *et al*., 1993; Parmar and Burka, 1993; Carfagna *et al*., 1993; Chen *et al*., 1995; Peterson *et al*., 2005; Peterson, 2006) (Figure 5). CYP 2E1 is known to metabolise and bioactivate multiple small-molecular weight toxicants such as ethanol, benzene, toluene and nitrosamines (Lieber, 1997). Other CYP P450 enzymes may also play roles in the bioactivation and/or metabolism of furan (Peterson *et al*., 2005). Furan metabolites such as BDA are known as reactive and cytotoxic. Only the bioactivated furan metabolites bind to proteins and nucleosides (Burka *et al*., 1991; Byrns *et al*., 2002; Peterson *et al*., 2011). BDA is formed by oxidation of one of the furan's double bonds, possibly with the formation of an epoxide intermediate that is spontaneously rearranged and opened. The reaction may involve the successive electron and oxygen transfers from a CYP P450-perferryl intermediate [FE(V)O]. BDA is stabilized in *cis*-configuration by a cyclic hydrate (Chen *et al*., 1995).

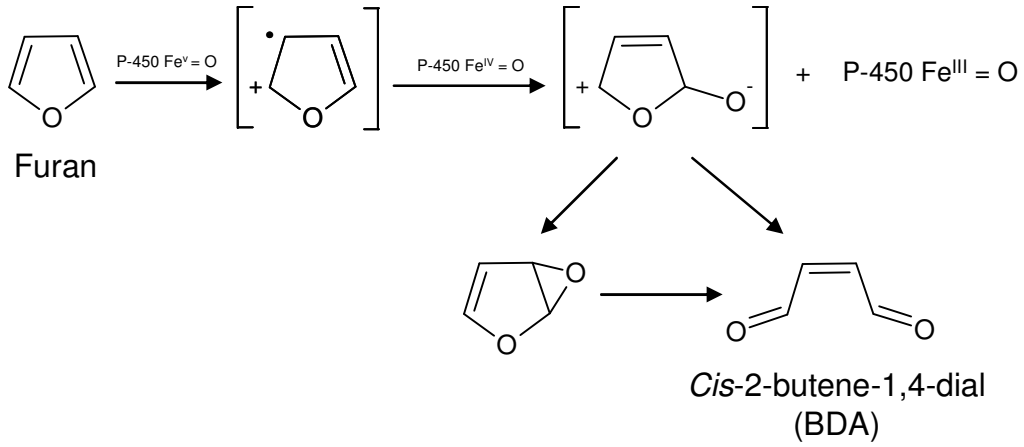

Figure 5: CYP-mediated bioactivation of furan to form reactive metabolite. Possible reaction intermediates from metabolism of furan by cytochrome P-450 (Parmar and Burka, 1993).

BDA reacts with glutathione (GSH) to a cyclic mono-glutathione conjugate that has been detected in the urine of furan-treated rats beside 18 additional minor metabolites (Burka *et al*., 1991; Peterson *et al*., 2006). GSH-BDA (2-(*S*-gluthathionyl)-succinaldehyde) has been shown to form pyrrole cross-links with biogenic amines such as lysine (N-acetyllysine conjugate, N-acetylcysteine-N-acetyllysine conjugate and its sulfoxide), glutamine (glutamic acid methanethiol), spermidine and ornithine. Their detection in primary hepatocytes and rat urine may indicate an important role in the toxicity of furan (Peterson *et al*., 2006; Kellert *et al*., 2008a; Lu *et al*., 2009; Hamberger *et al*. 2010; Peterson *et al*., 2011). However, conjugates apart from GSH-BDA have not yet been extensively studied.

Rat hepatic microsomes incubated with radio-labelled furan in the presence of NADPH electron generating system resulted in the covalent incorporation of furan-derived radioactivity in microsomal proteins. After oral application of 8 and 25 mg/kg bw furan to rats, decrease in cytochrome P450 (CYP) content and several CYP-dependent activities such as aniline hydroxylase (AH), 7-ethoxycoumarin-O-deethylase (ECOD), and 7-ethoxyresorufin-O-deethylase (EROD) was observed suggesting that the reactive furan intermediate can bind to nucleophilic groups of the P450 enzymes (Parmar and Burka, 1993).

Physiologically-based pharmacokinetic modelling (PBPK) including data from metabolism studies using primary hepatocytes from human and rodents in solution or culture has indicated that furan metabolism is rate-limited by the hepatic blood flow (Kedderis *et al*., 1993; Kedderis and Held, 1996). Therefore, induction of the main metabolizing enzyme CYP 2E1 would probably not increase the rate of furan metabolism (Kedderis and Held, 1996). However, inter-individual differences in the hepatic CYP 2E1-activity could have an influence on the extent of first-pass metabolism and bioactivation of furan. CYP 2E1 can be induced by ethanol and chronic ethanol consumption is known to increase the rate of metabolism (Chang and Kam, 1999). In contrast, acute ethanol consumption may competitively inhibit 2E1 and decrease metabolism. However, it is unlikely that CYP2E1 variations could considerably alter furan metabolism. Any increase in CYP 2E1 activity would not affect furan metabolism because it is the hepatic blood flow that is rate-limiting, not the CYP 2E enzyme activity. Only a substantial activity decrease could potentially reduce the formation of the noxious BDA.

CYP 2E1 demonstrates genetic polymorphism affecting the noncoding region of the gene, which may influence the transcription and expression of the gene (Chang and Kam, 1999). However, it has been shown for e.g. trichloroethylene that metabolism was altered with less than 2 % under extreme values of CYP2E1 expression and activity (Lipscomb *et al*., 2003). Human hepatic CYP2E1 expression varies during human fetal liver development and in the early postnatal stage, but already in 90 days old infants the activity level was comparable to that in children and adults (Johnsrud *et al*., 2003).

4.1.4 Elimination

After a single oral dose of 8 mg/kg bw radiolabelled furan to rats, 80% of the total radioactivity was eliminated via lung (40 %), urine (20 %), and faeces (22 %) within 24 hours (Burka *et al*., 1991). The expired air contained 14 % unchanged furan and 26 % carbon dioxide (as a product from furan metabolism). The furan radioactivity in the urine (20 %) was a sum of more than 10 metabolites.

Repeated dosing over 8 days resulted in the increase of excreted total radioactivity via urine to 33% whereas the fraction eliminated via the faeces remained approximately constant. However, the amount of tissue-bound radioactivity increased in liver (4-fold) and kidneys (6-fold) (Burka *et al*., 1991).

Pharmacokinetic data such as clearance, volume of distribution or half-life derived from *in vivo* studies are not yet available for furan. However, preliminary elimination parameters have been predicted on the basis of *in vitro* studies in rodent and human primary hepatocytes (Kedderis and Held, 1996). It was observed that the initial liver rate of furan metabolism in mouse, rat and human was approximately 13-, 24-, and 37-fold, respectively, greater than the respective hepatic blood flows (5.4 l/(h*kg), 4.2 l/(h*kg), and 1.4 l/(h*kg)). The maximal reaction velocity (V_{max}) (48, 18, and 19-44 nmol/(h*10^6 hepatocytes)) and reaction constant (K_M) (1.0, 0.4 and 2.1-3.3 µM) were determined for the three species (Table 32).

Hepatic enzyme activity can be determined as ratio of V_{max}/K_M provided that the substrate concentration in the assay is well below the K_M-value. The experimentally measured $CL_{int,assay} = V_{max}/K_M$ has to be upscaled to assay-independent, intrinsic liver clearances (CL_{int}) by considering the number of hepatocytes per g liver (hepatocellularities: HPGL) and the relative liver weight per kg body weight (RLW). This results in $CL_{int} = CL_{int,assay} * HPGL * RLW$ if protein binding to hepatocytes in the assay is negligible. *In vitro in vivo* extrapolation (IVIVE) can be performed by applying the well-stirred liver model calculating the systemic blood clearance (CL_b) from the CL_{int} by considering the hepatic blood flow (Q) according to ($CL_b = Q * Cl_{int}/(Q + Cl_{int})$ and assuming that protein binding of the furan molecule in plasma is irrelevant.

Table 32: Estimated kinetic parameters of furan, derived from in vitro metabolism (Kedderis and Held, 1996).

Parameter	Mouse	Rat	Human
V_{max} [nmol/(h*10^6 hepatocytes)]	48	18	19-44
$K_{M,assay}$ [µM]	1.0	0.4	2.1 – 3.3
$Cl_{int,assay}$ [l/(h*10^6 hepatocytes)]	0.048	0.045	0.009-0.013
HPGL [hepatocytes/g liver]	*170*10^6*	163*10^6	99*10^6
RLW [g liver/kg bw]	*50*	40	21
Cl_{int} [l/(h*kg)]	408	293	19 - 27
Q [l/(h*kg)]	5.4	4.2	1.4
Cl_b [l/(h*kg)]	~ 5.4	~ 4.2	~ 1.4

V_{max} - maximal reaction velocity, KM - reaction constant, CL_{int} - intrinsic liver clearances, HPGL – hepatocellularities, RLW - relative liver weight per kg body weight, Q - hepatic blood flow, CL_b - systemic blood clearance.

The calculated blood clearances for the different species approximate the respective hepatic blood flows, thus reaching the limits of the mathematical model. The *in vivo* blood clearances are equal to or exceeding the hepatic blood flow in the different species.

4.1.5 Summary of toxicokinetics

In conclusion, furan can pass through biological membranes and is rapidly absorbed from the lung and intestine. Systemic distribution is limited by high hepatic CYP 2E1-catalyzed elimination in high-capacity first-pass metabolism. Age-dependent expression of CYP 2E1 or polymorphisms have little impact on the furan metabolism. Oral exposure of up to 8 mg/kg bw/day resulted in furan liver levels of less than 1 ppb in rats. The amount of furan in the liver increased with the dose. The major metabolite BDA binds irreversible to proteins and nucleophiles. BDA can form conjugates *in vivo*, which have been little studied so far. Furan and furan-derived metabolites are eliminated via exhaled air, urine and faeces.

4.1 General toxicity

The general toxicity of furan has been described in several studies, and previously reviewed by EFSA (2004) and by Bakhiya and Appel (2010). The present overview is based on these

published reviews, NTP reports and some recent data (Gill *et al*., 2010). Only repeated and multiple dose toxicity studies with oral route of exposure have been included.

4.2.1 Short-term toxicity

4.2.1.1 Mice

B6C3F1 mice (n=5/dose/sex) were given furan in a 16 days dose-range study at 0, 10, 20, 40, 80 and 160 mg/kg bw in corn oil by gavage five days a week for 12 days, and at least two consecutive days before terminal sacrifice. The mice were weighted at the start of the study and at days 8 and 16. Clinical and toxicological observations were made twice daily. No histopathology was performed. Three male mice receiving 40 mg/kg bw and all male mice receiving 80 and 160 mg/kg bw did not survive beyond day 6. Four females receiving 80 mg/kg bw and all female mice receiving 160 mg/kg bw did not survive beyond day 6. Final mean body weight of males receiving 10 and 20 mg/kg were significantly greater than controls. Reduced activity was the most consistent clinical finding observed among furan-exposed mice. No observations at necropsy were clearly associated with furan treatment. No NOAEL could be allocated from this study (NTP, 1993).

Hepatocyte proliferation, apoptosis and levels of liver enzymes in serum were analysed in female B6C3F1 mice (n=6-11/dose) exposed to 0, 4, 8 and 15 mg/kg bw 5 days a week for 3 weeks. A statistical significant increase (2-3 fold) in serum liver enzymes (alanine aminotransferase (ALT) and sorbitol dehydrogenase (SHD)) was observed at 8 and 15 mg/kg bw doses. A dose-dependent increase in proliferation, measured by labelling index, was observed at all doses, and the apoptotic index increased 6- and 15-fold in mice receiving 8 and 15 mg/kg bw, respectively. VKM allocated a NOAEL of 2.85 mg/kg bw/day (adjusted to a daily exposure) based on liver toxicity giving > 2-fold increase in ALT and SHD (Fransson-Steen *et al.*, 1997).

Female B6C3F1 mice (n=15/dose) were exposed to furan at 0, 0.5, 1, 2 and 8 mg/kg bw in corn oil, 5 days a week for 3 weeks. Clinical chemistry, and cell proliferation and histopathology in the liver were examined. No treatment-related mortalities were observed during the study, and no treatment-related gross lesions were noted at the 3-week necropsy. A statistically significant increase in relative liver weight was observed in mice exposed to 8 mg/kg bw. A dose-dependent significant increase in hepatotoxicity and ALT levels in serum was observed in mice exposed to 1 mg/kg bw and higher dose levels compared to controls. A respective significant increase in ALT levels of 1.13-, 1.58-, 2.9- and 4.7-fold were observed at doses 1.0, 2.0, 4.0 and 8.0 mg furan/kg bw. Increased hepatocyte proliferation was observed in the 8 mg/kg bw dose group (Moser *et al*., 2009). VKM allocated a NOAEL of 1.42 mg/kg bw/day (adjusted to a daily exposure) based on liver toxicity giving > 2-fold increase in ALT.

Male B6C3F1 mice (n=6/dose from the text or n=7-11/dose from the Table, n=8/control) were given furan in corn oil by gavage at doses 0, 2, 4, 8 and 15 mg/kg bw 5 days a week for 28 days. Mortality, clinical signs and body weight were recorded, and histopathology, cell proliferation and apoptosis of the liver were reported. No mortality related to treatment was observed. Body weight or body weight gain was not significantly affected by treatment. A slight statistical significant increase in relative liver weight was observed in mice treated with 4 mg/kg bw furan and above. At the two highest furan doses, liver necrosis was observed that were suggested to be related to regenerative hyperplasia. A statistical significant, but not dose-related, increase in cell proliferation was observed at the two highest furan doses. An increased incidence of apoptotic cells was reported at the highest furan dose, but no

quantitative data were given. No NOAEL could be allocated due to liver necrosis at the two highest furan doses (Cordelli *et al*., 2010).

4.2.1.2 Rats

F344 rats (n=5 /dose/sex) were given furan in a 16 days dose-range study at 0, 5, 10, 20, 40 and 80 mg/kg bw (males) and 0, 10, 20, 40, 80 and 160 (females) in corn oil by gavage five days a week for 12 days, and at least two consecutive days. The rats were weighted at the start of the study and at days 8 and 16. Clinical and toxicological observations were made twice daily. All rats receiving ≥ 80 mg/kg bw of furan did not survive beyond day 8 of the study. Body weights of males receiving 20 or 40 mg/kg bw and females receiving 40 mg/kg bw were lower than those of control rats at the end of the study. Reduced activity was the only clinical finding consistently observed in the study, but the magnitude of this effect on the different dose levels was not given. No other significant findings were reported. No NOAEL could be allocated (NTP, 1993).

4.2.2 Sub-chronic toxicity

4.2.2.1 Mice

No treatment related mortalities were observed in B6C3F1 mice (n=10/dose/sex) given furan by gavage at doses 0, 2, 4, 8, 15, 30 and 60 (females only) mg/kg bw 5 days a week for 13 weeks. Final mean body weights of male mice receiving 30 mg/kg bw were lower than control mice. The absolute and relative liver weights at the two top dose levels (15 and 30 mg/kg bw in male mice, and 30 and 60 mg/kg bw in female mice) showed a dose-dependent significant increase compared to controls. A weak dose-dependent incidence of liver lesions was observed at 8 mg/kg bw in male mice and at 15 mg/kg bw in female mice, and increasing with dose, but reached statistical significance first at 30 mg/kg bw for both sexes. Nearly all animals (9-10 animals) developed liver lesions at 30 and 60 mg/kg bw. Clinical chemistry were not examined (NTP, 1993). VKM allocated a NOAEL of 10.7 mg/kg bw/day (adjusted to a daily exposure) for both sexes based on the increase in liver lesions.

Furan was given by gavage to F344 rats (n=10/dose/sex) at 0, 4, 8, 15, 30 and 60 (females only) mg/kg bw, 5 days a week for 13 weeks. Body weight was recorded weekly, and necropsies were performed on all animals at the end of the study. Complete histopathological examinations were made of all rats in the control group and on rats receiving 30 and 60 mg/kg bw. Nine male rats and four female rats from the 60 mg/kg bw dose group, did not survive to the end of the study. The final body weights of rats in the 15 and 30 mg/kg bw dose groups and remaining female rats in the 60 mg/kg bw dose group, were lower that the controls. In female rats, the relative and absolute liver and kidney weight were increased in the 15, 30 and 60 mg/kg bw dose groups, while the absolute and relative liver weights were increased and the thymus weight reduced in males receiving 30 mg/kg bw. Although, lesions associated with furan exposure were most pronounced in the liver, the kidney, thymus, testes and ovaries were also affected. The most prominent change was a dose-dependent statistically significant increase in the incidence of bile duct hyperplasia, which occurred in all furan-dosed groups in both sexes (NTP, 1993). A statistical significant increase in the incidence of cholangiofibrosis was observed at all furan doses in male rats, and at ≥ 8 mg furan/kg bw in female rats. Therefore, no NOAEL could be allocated from this study by VKM.

F344 rats (n=12 /dose/sex) were given furan at 0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw in corn oil by gavage, five days a week for 90 days. Animals were observed for clinical signs twice daily/week and once daily/weekends. Food consumption was measured weekly. At the

end of the study, the animals were sacrificed and blood samples were collected for measurements of haematology and clinical biochemistry. Necropsies were performed and liver, kidneys, thymus, uterus ovaries, ventral prostate, seminal vesicles and testes were weighted. A dose-related statistically significant increase in platelet count was observed at ≥ 0.5 mg/kg bw in females, and at 8 mg/kg bw in males. Dose-related changes in clinical chemistry were noted for several parameters related to liver function. A significant change in clinical chemistry was observed from ≥ 2 mg furan/kg bw in females and from ≥ 0.12 mg furan/kg bw in males on thyroxine (T4) and triiodothyronine (T3), while other clinical parameters were increased from ≥ 2.0 mg furan/kg bw. A significant reduction in liver enzymes (alanine transaminase, alkaline phosphatise, aspartate transaminase) was observed in male and female rats from ≥ 2 mg/kg bw. The absolute liver weight was increased at the highest dose tested in both sexes. Macroscopic changes were observed in all of the livers of both males and females in this dose group. Histological lesions of the liver were observed in male and female rats, starting at 0.12 mg furan/kg bw. The severity and number of lesions increased with increasing furan dose. Increased incidence of cholangiofibrosis (17% and 100%) and biliary tract hyperplasia (50% and 100%) were observed in the two highest dose groups for males increasing with the dose, and in the highest dose group for female rats (100% and 92% incidence for hyperplasia and cholangiofibrosis, respectively) (Gill *et al*., 2010). An overview of the incidences of biliary toxicity in rats reported in this study is illustrated in Table 33. VKM allocated a NOAEL of 0.36 mg/kg bw/day (adjusted to a daily exposure) for both sexes based on the increase in hyperplasia and cholangiofibrosis in liver.

Table 33: Incidences of biliary toxicity in rats (Gill *et al*., 2010)

Biliary tract toxicity	**Females**						**Ref.**
	Control	**0.03 mg/kg**	**0.12 mg/kg**	**0.5 mg/kg**	**2 mg/kg**	**8 mg/kg**	
Hyperplasia	0/12	0/12	0/12	0/12	1/12	12/12	Gill *et al*., 2010
Cholangiofibrosis	0/12	0/12	0/12	0/12	0/12	11/12	Gill *et al*., 2010
Biliary tract toxicity	**Males**						**Ref.**
	Control	**0.03 mg/kg**	**0.12 mg/kg**	**0.5 mg/kg**	**2 mg/kg**	**8 mg/kg**	
Hyperplasia	0/12	0/12	0/12	0/12	6/12	12/12	Gill *et al*., 2010
Cholangiofibrosis	0/12	0/12	0/12	0/12	2/12	12/12	Gill *et al*., 2010

4.2.3 Chronic toxicity

4.2.3.1 Mice

B6C3F1 mice (n=50/dose/sex) were given furan in corn oil by gavage at doses of 0, 8 and 15 mg/kg bw five days a week for 2 years. The mean body weights of both male and female mice in the high-dose group were lower than in the control group. From week 80 to termination of the study, survival of the males in both dose groups and females in the high dose group were lower than in the control group. The incidences of numerous non-neoplastic hepatocellular lesions were also increased in furan treated mice. These lesions included hepatocyte cytomegaly, degeneration, necrosis, multifocal hyperplasia, cytoplasmic vacuolization and

biliary tract dilatation, fibrosis, hyperplasia, and inflammation, and were significant for nearly all effects at both doses for both male and females. No NOAEL could be allocated from this study (NTP, 1993).

Groups of female B6C3F1 mice were given furan by gavage at 0 (n = 50), 0.5 (n = 100), 1.0 (n = 75), 2.0 (n = 50), 4.0 (n = 50) and 8.0 (n = 50) mg/kg bw five days a week for 2 years. Complete gross examination and macroscopic examination of the liver was performed at necropsy. There was no significant difference in the percentage of mice surviving at the termination of the study. In mice exposed to 2.0 and 8.0 mg furan/kg bw, there was a significant decrease in body weight gain from study initiation to terminal necropsy, but this decrease was not dose-related. In mice exposed to 4.0 and 8.0 mg furan/kg bw, absolute and relative liver weight were significantly increased, and a significant increase in gross liver nodules were observed at the same doses. A NOAEL for general toxicity of 2.0 mg/kg bw/day was allocated based on the increase in liver weight and gross liver nodules (Moser *et al*., 2009).

4.2.3.2 Rats

F344 rats (n=70/dose/sex) were given furan by gavage at 0, 2, 4 and 8 mg/kg bw in corn oil for 5 days a week in 2 years. After dosing for 9 and 15 months, 10 rats from each group were evaluated for the presence of treatment-associated lesions. Mean body weight and survival were reduced in rats receiving 8 mg furan/kg from week 73 and 85, respectively. The most marked dose-related effects were observed in the liver. Non-neoplastic liver lesions were abundant in all rats administered furan at all doses, with the highest toxicity in the biliary tract in the liver. All effects in the biliary tract like; cyst, focal fibrosis, hyperplasia and metaplasia, occurred with an incidence of 88% and 98% at the lowest dose in male and female rats respectively. Non-neoplastic toxicity in the hepatocytes like degeneration, hyperplasia, necrosis and pigmentation occurred with an incidence of 36% to 98% at the lowest dose. Non-neoplastic lesions were also observed in the hematopoietic system, like pancreatic lymph node, spleen and the bone marrow. No NOAEL could be allocated for chronic toxicity from this study. Since nearly 100% incidence of biliary tract toxicity are observed at the lowest dose, the data are not suitable for BMD calculations (NTP, 1993).

4.2.4 Summary of general toxicity

Furan exposure showed a dose-related toxicity in both mice and rats. The most critical toxicological effects were observed in the liver in both species, but rats were more sensitive to furan toxicity than mice. Non-neoplastic liver lesions were abundant in all rats administered furan at all doses in the chronic toxicity study, with the highest toxicity in the biliary tract in the liver.

4.3 Genotoxicity

An overview of the genotoxicity studies on furan and its metabolite *cis*-2-butene-1,4-dial (BDA), with corresponding tables is presented in the following sections.

4.3.1 In vitro

4.3.1.1 Furan

The studies commented on below are summarised in Table 34.

Furan did not induce gene mutations when tested in the *Salmonella typhimurium* strains TA100, TA1535, TA1537 and TA98 either with or without S9-mix when tested with 33-10.000 µg/plate (Mortelmans *et al*., 1986 cited in NTP, 1993).

A concentration-dependent increase (at concentrations from 1100 to 3800 µg/ml) in gene mutations was found in the mouse lymphoma assay in the absence of S9-mix (McGregor *et al*., 1988, cited in NTP, 1993).

Kellert *et al*., (2008b) could not reproduce this positive result for gene mutations in the mouse lymphoma assay without S9-mix. In the same study furan did not induce strand breaks or micronuclei in mouse lymphoma cells at effective concentrations (i.e. after corrections for decreased furan concentrations as measured by evaporation and diffusion of test materials in water) of 0, 0.225, 0.45, 0.9, 1.6 and 3.1 mM. No metabolic activation was used for all genetic end points. In the same study, BDA was also investigated for genotoxic effect (see section 4.3.1.2).

At concentrations from around 5 to 200 mM (or 340 to 13614 µg/ml) furan induced a concentration-dependent increase in chromosomal aberrations (mainly exchanges) in Chinese hamster ovary (CHO) cells in the presence, but not in the absence of Aroclor 1254 induced rat liver S9 mix (Stich *et al*., 1981).

Furan (tested up to 500 µg/mL) was weakly positive in a sister chromatid exchanges (SCE) test in CHO cells both in the absence and presence of S9 activation (Galloway, 1985; 1987 cited in NTP, 1993), while it induced a concentration-dependent increase (when tested up to 1000 µg/mL) in chromosomal aberrations in CHO cells both with and without S9 mix (Galloway, 1985; 1987 cited in NTP, 1993).

Negative results were obtained in a micronucleus assay in human lymphocytes both with and without S9-mix when tested up to 100 mM (or 6805 µg/ml) (Durling *et al*., 2007).

Exposure of rat and mouse hepatocytes to 10 mM (or 681 µg/ml) furan did not induce unscheduled DNA synthesis as an indicator of DNA damage (Wilson *et al*., 1992). This study was performed in conjunction with an *in vivo* UDS assay and the *in vitro* data was not shown. No metabolic activation was used.

Furan was negative in the sex linked recessive lethal assay in Drosophila melanogaster (NTP, 1993; Foureman *et al*., 1994).

In addition to these studies recent data was found in a summary of the EU project "Role of genetic and non-genetic mechanisms in furan risk" (Dekant *et al*., 2007). These studies are not yet published and therefore used only as supplementary data. They are summarised below, but not included in Table 34.

Furan and its metabolite BDA (see below) were tested for gene mutations in V79 and L5178Y cells at the *hprt* and *tk* locus, respectively. Furan itself was not mutagenic in either test at

concentrations up to the highest recommended concentration of 10 mM using 3 or 24 h exposure (Dekant *et al*., 2007).

Furan was tested at 1.6, 2.5, 4.0, 6.3 and 10 mM in the absence and in the presence of optimized metabolic activation (CYP2E1) (Dekant *et al*., 2007). Different cell lines were used: "normal" lymphocytes and two lymphoblastoid cell lines from Fanconi´s anemia patients, the latter being special sensitive to DNA crosslinking substances. In normal lymphocytes furan did not induce chromosomal aberrations after 3 h exposure and 24h sampling time either with or without metabolic activation (CYP2E1). Surprisingly, there was an increase in chromosomal aberration without S9 after prolonged exposure times 24 h and 44 h at the highest concentrations tested. The positive result without metabolic activation was unexpected and explained by the study authors as autoxidation of furan to DNA reactive metabolites (Mosesso, 2009). In Fanconi´s anemia cells furan induced chromosomal aberrations in the presence of optimized S9, and to a lesser extend also without S9 (Dekant *et al*., 2007).

4.3.1.2 Cis-2-butene-1,4-dial (BDA)

The studies commented on below are summarised in Table 35.

In accordance with the possible role of *cis*-2-butene-1,4-dial (BDA) in furan toxicity and genotoxicity, it has been shown that this metabolite reacts rapidly with model protein and nucleic acids to form adducts (Chen *et al*., 1997). BDA was directly mutagenic in the *S. typhimurium* strain TA104, a strain sensitive to aldehydes and able to detect DNA-crosslinks, but negative in TA98, TA97, TA100 and TA102 strains (Peterson *et al*., 2000).

In mouse lymphoma cells, BDA (6.3-50 µM) induced gene mutations at the *tk* locus and DNA strand breaks in a concentration-dependent manner using the alkaline Comet assay, but it did not induce micronuclei (Kellert *et al*., 2008b).

BDA induced DNA single-strand breaks and cross-links in CHO cells (Marinari *et al*., 1984). More recent *in vitro* studies have shown that BDA produced DNA adducts (Byrns *et al*., 2002; Bohnert *et al*., 2004).

In studies related to the EU project "Role of genetic and non-genetic mechanisms in furan risk" (Dekant *et al.,* 2007), BDA was tested for gene mutations in mammalian cells (V79 and L5178Y cells at the *hprt* and *tk* locus, respectively) in parallel with furan. BDA was highly cytotoxic and induced mutations in both assays but in a very narrow concentration range. In the TK assay there was an increase in the relative number of small colonies indicating a clastogenic activity. This mode of action was confirmed by a positive response in a micronucleus assay in the same concentration range. BDA was only clastogenic in "normal" lymphocytes after 24h exposure, but was highly clastogenic in Fanconi´s anemia cell lines in a narrow concentration range.

4.3.1.3 Summary of in vitro studies

Furan is metabolised to a DNA reactive metabolite, BDA, by hepatic cytochromes P450 enzymes (CYPs), predominantly CYP2E1. Furan and BDA have been tested in several *in vitro* genotoxicity tests using both bacteria and mammalian cells. BDA was found positive in the vast majority of studies, except for one inadequately performed micronucleus assay, whereas conflicting results have been obtained for furan, with many negative results in standard assays.

The *in vitro* genotoxicity of compounds like furan (and acrylamide) that require CYP2E1 metabolism for activation is difficult to detect in standard *in vitro* genotoxicity assays due to inadequate metabolic activation systems.

The negative results obtained with furan with standard S9 activation; do therefore not reflect absence of genotoxicity of furan via BDA, but rather a limitation of "conventional" genotoxicity assays to detect such compounds. When S9 with a high content of CYP2E1 was used in test systems, which can detect crosslinking substances, positive results for furan were obtained.

Positive results have been obtained with furan without metabolic activation, which could be caused by artefacts due to the extremely high concentrations used in older studies, or because of an autooxidation of furan to DNA reactive substances after long exposure time *in vitro*. These results are not considered to be of biological relevance by VKM.

Table 34: Genotoxicity of furan - *In vitro* studies

Test system	Test object	Concentration	End point	Results	Comments from VKM	Ref.
Ames test	*S. typhimurium* TA100, TA1535, TA1537, TA98	33, 100, 333, 1000, 3333, 10000 µg/plate	Gene mutations	Negative (+S9) Negative (-S9)	These strains cannot detect cross-linking agents. No appropriate metabolic activation	NTP, 1993 Mortelmans *et al*., 1986
Mouse lymphoma $tk^{+/-}$ assay	Mouse L5178Y lymphoma cells	**Trial 1**:125, 250, 500, 1000, 2000 µg/mL **Trial 2**: 1400, 2000, 2600. 3200, 3800 µg/mL **Trial 3:** 1139, 1627, 2116, 2604, 3090 µg/mL	Gene mutations and to some extent chrom. ab.	Positive (-S9) (+ S9 – not tested)	**Trial 1**: No effect. **Trial 2**: a concentration-dependent increase at the two highest concentrations. **Trial 3**: a concentration-dependent increase at all concentrations tested. LOED = 1139 µg/mL (=16.8 mM) These results are not considered biologically relevant because no metabolic activation was used	NTP, 1993 McGregor *et al*., 1988
Mouse lymphoma $tk^{+/-}$ assay	Mouse L5178Y lymphoma cells	0, 0.225, 0.45, 0.9, 1.6 or 3.1 mM (=211 µg/mL)	Gene mutations and to some extent chrom. ab.	Negative (no S9 mix used)	Evaporation of furan in water measured, and furan conc. adjusted. No metabolic activation was used, in order to compare with previous positive results without S9 mix (NTP 1993 and McGregor *et al.* 1988). In the same study BDA was also tested.	Kellert *et al*., 2008b
Chromosomal aberration assay	Chinese hamster ovary cells	5 to 200 mM (340- 13610 µg/mL)	Structural chrom. ab.	Positive (+S9) (mainly exchanges) in the range from 100 to 200 mM (= 6805 to 13610 µg/mL) Negative (-S9)	A concentration-dependent increase in aberrations, at extremely high concentration Results only shown in small figures, without exact concentrations. Invalid study, positive only at concentrations above the highest recommended concentration of 10 mM	Stich *et al*., 1981
Chromosomal aberration assay	Chinese hamster ovary cells	0, 160, 300, 500, 1000 µg/mL (+S9) 0, 100, 160, 300, 500 µg/mL (-S9)	Structural chrom. ab.	Positive (+S9) Positive (–S9)	Positive at the two highest concentrations +S9 (1 test) Positive in two tests – S9 at all concentrations with a concentration related response , but not considered biologically relevant	NTP, 1993
Micronucleus assay	Human lymphocytes	**Trial 1**: 0, 2, 5, 7.5, 10, 15, 20 or 100 mM (=6805 µg/mL) **Trial 2**: 5, 10, 17.2 or 25 mM (=1701 µg/mL)	Structural and numerical chrom. ab.	Negative (+S9) Negative (-S9)	Strong cytotoxicity was observed at the highest dose in both trials, i.e. 25 and 100 mM	Durling *et al*., 2007
Micronucleus assay	Mouse L5178Y lymphoma cells	0, 0.225, 0.45, 0.9, 1.6 or 3.1 mM (=211 µg/mL)	Structural and numerical chrom. ab.	Negative (no S9 mix used)	Evaporation of furan in water measured, and furan concentration adjusted. No metabolic activation was used. In the same study BDA was also tested	Kellert *et al*., 2008b
Sister chromatid	Chinese hamster ovary	0, 16, 50, 160 and 500 µg/mL (+S9)	Chromatid exchanges	Weak positive (+S9) Weak positive (-S9)	Positive in all concentrations first trial (weak concentration response) but only at the highest concentration in second	NTP, 1993

Test system	Test object	Concentration	End point	Results	Comments from VKM	Ref.
exchange test	cells	0, 5, 50 and 160 µg/mL (-S9)			trial–S9. Not considered biologically relevant	
Comet assay	Mouse L5178Y lymphoma cells	0, 0.225, 0.45, 0.9, 1.6 or 3.1 mM (=211 µg/mL)	Primary DNA damage leading to strand breaks	Negative (no S9 mix used)	Evaporation of furan in water measured, and furan concentration adjusted. No metabolic activations used. In the same study BDA was also tested	Kellert *et al*., 2008b
Unscheduled DNA synthesis	Mouse and rat hepatocytes	No doses given	Indirect measure of DNA damage	Negative (no S9 mix used)	Evaporation of furan in water measured, but furan concentration NOT adjusted. Data not shown. No metabolic activation used	Wilson *et al*., 1992

Ref. – reference, chrom. ab. - chromosomal aberration, conc. – concentration, LOED – lowest observed effect dose

Table 35: Genotoxicity of cis-2-butene-1,4-dial (BDA) - *In vitro* studies

Test system	Test object	Concentration	End point	Results	Comments from VKM	Ref.
Ames test	TA98, TA 97, TA100, TA102 and TA104	1.4, 1.7, 2.1, 2.9, and 4.4 µmol/plate (=95, 116, 143, 197, 299 µg/plate)	Gene mutation	Positive in TA104 –S9 Negative in TA98, TA 97, TA100 and TA102	Potent inducer of gene mutation with a concentration related increase up to 2.1 µmol/plate, and toxic at higher concentrations. This study indicates that BDA can form DNA-DNA cross-links. Mutagenicity was only detected in a narrow concentration range	Peterson *et al*., 2000
Mouse lymphoma $tk^{+/-}$ assay	Mouse L5178Y lymphoma cells	6.3, 12.5, 25, 50 µM (= 0.4, 0.9, 1.7 3.4 µg/ml)	Gene mutation and to some extend chrom. ab.	Positive at all concentrations (-S9)	A clear and concentration related increase at all concentrations. A strong cytotoxic effect observed at 50 mM (RTG=2%) at 25mM RTG =36% Three independent assays performed with the same results. The assay was performed together with a Micronucleus assay and a Comet assay with BDA and furan	Kellert *et al*., 2008b
Micronucleus assay	Mouse L5178Y lymphoma cells	6.3, 12.5, 25, 50 µM (= 0.4, 0.9, 1.7 3.4 µg/ml)	Structural and numerical chrom. ab.	Negative	The assay was performed together with a gene mutation test and a Comet assay with BDA and furan. Inadequate protocol, longer treatment time should have been included	Kellert *et al*., 2008b
Comet assay **1.** Standard alkaline **2.** Modified with gamma radiation after BDA treatment	Mouse L5178Y lymphoma cells	6.3, 12.5, 25, 50 µM (= 0.4, 0.9, 1.7 3.4 µg/ml)	DNA strand breaks and DNA cross-links	1. Positive for strand breaks 2.No indication of formation of DNA cross-links	The assay was performed together with a gene mutation test and a Micronucleus assay with BDA and furan	Kellert *et al*., 2008b

Test system	Test object	Concentration	End point	Results	Comments from VKM	Ref.
Alkaline elution with and without exposure to MMS	CHO-K1 cells	0.17, 0.5 and 1.5 mM	DNA breaks and DNA cross-links	Positive for strand breaks; increase in elution without MMS Positive for cross-links; decrease in elution with MMS		Marinari *et al*., 1984
Mouse lymphoma $tk^{+/-}$ assay	Mouse L5178Y lymphoma cells	Not given 3 and 24 h exposure	Genmutations and to some extend chrom. ab.	Positive in a narrow concentration range close to cytotoxic concentrations. Increase in small colonies	BDA was tested in parallel with furan. Summary report only	Dekant *et al*., 2007
Gene mutation at the *hprt* locus	V79 cells	Not given 3 and 24 h exposure	Gene mutations	Positive in a narrow concentration range close to cytotoxic concentrations	BDA was tested in parallel with furan. Summary report only	Dekant *et al*., 2007
Micronucleus assay	Mouse L5178Y lymphoma cells	Not given 3 and 24 h exposure	Structural and numerical chrom. ab.	Positive	BDA was tested in parallel with furan. Summary report only	Dekant *et al*., 2007
Chromosomal aberration test	"Normal human lymphocytes"	1.6, 2.5, 4.0, 6.3 and 10 mM 3 and 24 h exposure	Structural chrom. ab.	Positive but only after long treatment (24h)	BDA was tested in parallel with furan. Summary report only	Dekant *et al*., 2007
Chromosomal aberration test	Lymphoblastoid cell line from Fanconi´s anaemia patients	3 and 24 h exposure	Structural chrom. ab.	Highly clastogenic	BDA was tested in parallel with furan. Summary report only. This cell line is known to be special sensitive to DNAcross-linking agents	Dekant *et al*., 2007

Ref. – reference, chrom. ab. - chromosomal aberration

4.3.2 In vivo

4.3.2.1 Furan

Rats

Liver

The studies commented on below are summarised in Table 36.

After single oral exposure at doses up to 200 mg/kg body weight in male Fischer 344 rats, furan did not induce unscheduled DNA synthesis in the liver (Wilson *et al*., 1992). However, the UDS assay only detects bulky DNA adducts and is considered to be insensitive to genotoxicity resulting from misrepair and non-repair, and therefore has been considered unsuitable to demonstrate the absence of genotoxicity of furan *in vivo* (Heppner and Schlatter, 2007; Leopardi *et al*., 2010).

In a very recent study (Neuwirth *et al*,. 2012) from the EU project "Role of genetic and non-genetic mechanisms in furan risk", it was reported that in male rats treated with [3,4-^{14}C]-furan at a known carcinogenic dose (2 mg/kg bw) and a dose closer to estimated human exposure (0.1 mg/kg bw) a significant, dose-dependent increase in the ^{14}C-content in the rat liver DNA was observed. In this study it was stated that the majority of the ^{14}C label was not associated with unmodified nucleotides, suggesting that the increase in ^{14}C-content in DNA extracted from livers of rats treated with ^{14}C-furan is primarily due to covalent binding to DNA rather than metabolic incorporation. Although, the structure of the furan-derived DNA adducts remains to be identified, this study indicates that furan metabolites can also react with DNA *in vivo* (Neuwirth *et al*., 2012). In the same study, DNA damage, measured by the Comet assay, was observed in the liver at the high dose.

In another recent study, McDaniel *et al*. (2012) tested furan in Big Blue rats. The rats were dosed 5 times a week for 8 weeks with 2 doses of furan used in the NTP cancer bioassay (2 and 8 mg/kg bw/day) and two higher doses (16 and 30 mg/kg bw/day). Several genetic end points were measured at different time points either in the liver or in blood/bone marrow (see below). In the liver, gene mutations were measured at the *cII* gene and DNA damage by the Comet assay. Furan induced DNA damage measured by the Comet assay occurred at the two highest doses in a dose-related manner, but gene mutations were not observed at the *cII* locus at any of the tested doses. Liver toxicity was observed at the highest tested dose (30 mg/kg bw). As discussed by the study authors the discrepancy between the results in these assays on the liver could be due to non-optimal sampling times (time between last treatment and time of sampling), which is later discussed in section 4.3.2.2.

Table 36: Genotoxicity of furan - *In vivo* studies in rat – Liver

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Unscheduled DNA synthesis	Male F-344 rats (n = 3) Gavage	5, 30 and 100 mg/kg bw	Indirect measure of DNA damage.	Negative	Limited relevance as assay only detects bulky DNA adducts	Wilson *et al*., 1992
DNA adducts in liver	Male F-344 rats Gavage	0, 0.1 and 2 mg/kg bw of [3,4-^{14}C]-furan	DNA adducts	Positive	Dose related increase in ^{14}C rat liver DNA	Neuwirth *et al*., 2012
Comet assay on liver	Male F-344 rats Gavage	0, 0.1, 0.5 and 2 mg/kg bw for 28 days (5 days per week)	Primary DNA damage leading to strand breaks	Positive	-	Neuwirth *et al*., 2012

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
cII gene mutation test on liver	Big Blue rats Gavage	2, 8, 16, 30 mg/kg bw for 8 weeks (5 days a week)	Gene mutations	Negative	To short expression time for the fixation of mutations	McDaniel *et al*., 2012
Comet assay on liver	Big Blue rats Gavage	2, 8, 16, 30 mg/kg bw for 8 weeks (5 days a week)	Primary DNA damage leading to strand breaks	Positive at the 2 highest (toxic doses)	To long sampling time for the Comet assay. Standard Comet assay cannot detect crosslinks	McDaniel *et al*., 2012

Ref. – reference

Spleen

In the study by Neuwirth *et al.* (2012), oral administration of furan to Fischer rats of 0, 0.1, 0.5 and 2 mg/kg bw/day by gavage for 4 weeks (5 days per week) induced significant increases of chromosomal aberrations in G_0 splenocytes after growth stimulation *in vitro* (see Table 37).

Table 37: Genotoxicity of furan - *In vivo* studies in rat – Spleen

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Chromosomal aberrations in G_0 splenocytes After growth stimulation *in vitro*	Male F-344 rats Gavage	0, 0.1, 0.5 and 2 mg/kg bw for 5 and 28 days (5 days per week)	Structural chrom. ab.	Positive	Accumulation in G_0 cells expression after growth stimulation *in vitro*	Neuwirth *et al*., 2012

Ref. – reference, chrom. ab. - chromosomal aberration

Bone marrow and peripheral blood

The studies commented on below are summarised in Table 38.

In the same study by Neuwirth *et al*. (2012), negative results were obtained for induction of micronuclei, chromosomal aberrations and sister chromatid exchanges and DNA strand breaks measured by the Comet assay in the bone marrow of male F344/N rats orally dosed with 0.1, 0.5 and 2 mg/kg bw/day for 5 and 28 days, followed by a 2 week recovery period. The study authors explained the negative results in the bone marrow by limited distribution of the furan-metabolite BDA to the bone marrow and a selective elimination of cells with damaged DNA due to cell proliferation.

In the Big Blue rat study by McDaniel *et al*. (2012), furan genotoxicity was also tested in the bone marrow and on peripheral blood. Twenty four hours after the 5th dose (one week of dosing) blood samples were taken and used to assay for micronucleus (MN) frequency in normachromatic erythrocytes (NCEs) and reticulocytes (RETs) and *Pig-a* gene mutations in total red blood cells (RBCs). Twenty four hours after the last dose of the 8-week treatment schedule, the rats were sacrificed and their tissues were used to perform NCE and RET MN assays, the *Pig-a* RBC assay, *Pig-a* and *hprt* lymphocyte gene mutation assay. Negative responses were obtained for all the gene mutation assays and the MN assays at both sampling times. As discussed by the study authors the negative result in the MN, *Pig-a* and *hprt assay*s could be explained by the fact that the putative genotoxic metabolite, BDA, is likely formed in the liver and due to its high reactivity will react with the liver constituents and not reach the bone marrow.

Table 38: Genotoxicity of furan - *In vivo* studies in rat – Bone marrow or peripheral blood

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Pig-a gene mutation test and *hprt* gene mutation assay on blood cells	Big Blue rats Gavage	2, 8, 16, 30 mg/kg bw for 8 weeks (5 days a week)	Gene mutations	Negative	Substance may not have reach the target organ	McDaniel *et al*., 2012
Micronuclei on blood cells and bone marrow	Big Blue rats Gavage	2, 8, 16, 30 mg/kg bw for 8 weeks (5 days a week)	Structural and numerical chrom. ab.	Negative	Substance may not have reach the target organ	Mc Daniel *et al*., 2012
Micronuclei, Chrom. ab. and SCE in bone marrow	Male F-344 rats Gavage	0, 0.1, 0.5 and 2 mg/kg bw for 5 and 28 days (5 days per week)	Structural and numerical chrom. ab. and chromatid exchanges	Negative	Substance may not have reach target organ	Neuwirth *et al*., 2012
Comet assay in bone marrow and blood cells	Male F-344 rats Gavage	2 mg/kg bw for 5 and 28 days (5 days per week)	Primary DNA damage leading to strand breaks	Negative	Substance may not have reach target organ	Neuwirth *et al*., 2012

Ref. – reference, chrom. ab. - chromosomal aberration

Mice

Liver

The studies commented on below are summarised in Table 39.

After oral exposure at single doses up to 100 mg/kg body weight in male B6C3F1 mice, furan did not induce unscheduled DNA-synthesis in the liver (Wilson *et al*., 1992). However, the UDS assay only detects bulky DNA-adducts and is considered to be insensitive to genotoxicity resulting from misrepair and non-repair, and therefore has been considered unsuitable for demonstrating the absence of genotoxicity of furan *in vivo* (Heppner and Schlatter, 2007, Leopardi *et al*., 2010).

In order to study the mode of action of the liver carcinogenicity of furan, Cordelli and co-workers (2010) investigated different end points, including genotoxicity, involved in the development of cancer in the liver of B6C3F1 mice in a 28-day repeated oral toxicity study using the same dose levels as in the NTP carcinogenicity study (2, 4, 8 and 15 mg/kg bw/day). No induction of DNA double-strand breaks, measured as λ-H2AX foci, increase in overall DNA methylation, or induction of DNA single-strand breaks or cross-links measured by the Comet assay after repeated exposure to relative low doses of furan were observed. However, there was an over-expression of several DNA repair genes, which according to the authors could be an indirect evidence of genotoxicity, also indicating that the Comet assay cannot detect furan induced DNA damage at low exposure levels.

In addition, there was a significant increase in polyploid (8N) and endo-reduplicated liver cells, which according to the study authors could be associated with accumulation of DNA damage.

In the same study by Cordelli *et al*. (2010), administration of a single dose of furan (15, 100, 250 mg/kg bw) gave an increase of DNA damage in the liver measured by the Comet assay at the highest toxic dose (250 mg/kg bw) and a distinct decrease in λ-ray DNA damage indicating the induction of DNA cross-links, possibly due to the metabolite BDA.

Table 39: Genotoxicity of furan - *In vivo* studies in mice – Liver

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Unscheduled DNA synthesis	Male B6C3F1/CrIBR mice (n = 1-4) Gavage	5, 50, 100 and 200 mg/kg bw	Indirect measure of DNA damage.	Negative	Limited relevance as assay only detects bulky DNA adducts	Wilson *et al*., 1992
Comet assay	Male B6C3F1 mice (n = 7-9) Gavage	0, 2, 4, 8 or 15 mg/kg bw/day for 28 days	Primary DNA damage leading to strand breaks	Negative (liver)		Cordelli *et al*., 2010
Comet assay	Male B6C3F1 mice (n = 5-6) Gavage	Single dose of 0, 15, 100 or 200 mg/kg bw	Primary DNA damage leading to strand breaks	Positive (liver)	Positive in liver at the highest dose, with liver toxicity	Cordelli *et al*., 2010
Comet assay (modified with γ-ray)	Male B6C3F1 (n = 5-6) Gavage	Single doses of 0, 15, 100 or 250 mg/kg bw	Cross binding of DNA	Positive (liver)	Positive in liver at the highest dose, with liver toxicity	Cordelli *et al*., 2010

Ref. – reference

Spleen

The studies commented on below are summarised in Table 40.

Due to the ability of splenocytes to accumulate DNA damage, the spleen was selected as the target organ for genotoxicity assessment in another *in vivo* genotoxicity study with furan by the same research group as above (Leopardi *et al*., 2010). Male B6C1F1 mice were given furan by gavage at doses 0, 2, 4, 8 or 15 mg/kg bw/day five days a week for 28 days. In another experiment by Leopardi *et al*. (2010), mice were exposed to single doses of 0, 15, 100 or 200 mg/kg bw of furan.

In mitogen-stimulated splenocytes a dose-dependent increase in micronucleated binucleate splenocytes was observed after 28 days of furan exposure. Also, an increase in induction of DNA double-strand breaks, measured as λ-H2AX foci, was observed at the two highest dose-levels. The authors concluded that these results indicate that furan exposure *in vivo* gives rise to pre-mutagenic lesions, which can be converted to chromosomal aberrations after growth stimulation. After acute exposure to much higher doses a treatment related trend in micronucleated binucleate splenocytes was observed, which, however, did not reach statistical significance.

In non-stimulated isolated splenocytes furan did not induce strand breaks in the Comet assay after both acute and 28 days furan exposure, which could be due to formation of DNA-DNA cross-links, which is not expected to be detected in a standard Comet assay. However, in a modified Comet assay no indication of DNA cross-linking was observed in splenocytes after γ-ray treatment (Leopardi *et al*., 2010).

Table 40: Genotoxicity of furan - *In vivo* studies in mice – Spleen

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Comet assay	Male B6C3F1 (n = 5-6) Gavage	0, 2, 4, 8 or15 mg/kg bw/day for 28 days	Primary DNA damage leading to strand breaks	Negative (splenocytes)		Leopardi *et al*., 2010
Micronucleus assay (splenocytes)	Male B6C3F1 (n = 5-6) Gavage	0, 2, 4, 8 or15 mg/kg bw/day for 28 days	Structural and numerical chrom. ab.	Positive (growth stimulated	Dose-dependent increase	Leopardi *et al*., 2010

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
and λ-H2AX foci				splenocytes)		
Comet assay	Male B6C3F1 (n = 5-6) Gavage	Single doses of 0, 15, 100 or 250 mg/kg bw	Primary DNA damage leading to strand breaks	Negative (splenocytes)		Leopardi *et al*., 2010
Comet assay (modified with γ-ray)	Male B6C3F1 (n = 5-6) Gavage	Single doses of 0, 15, 100 or 250 mg/kg bw	Cross binding of DNA	Negative (splenocytes)		Leopardi *et al*., 2010

Ref. – reference, chrom. ab. - chromosomal aberration

Bone marrow

The studies commented on below are summarised in Table 41.

Intraperitoneal injection of furan was given to male B6C3F1 mice. Bone marrow cells were analysed for chromosomal aberrations (CA) and sister chromatid exchange (SCE). Furan induced structural CA but not SCE after doses up to 350 mg/kg bw (trial 1, harvest after 17 hours) and after doses up to 250 mg/kg bw (trial 2 and 3, harvest after 36 hours) (NTP, 1993). Durling *et al*. (2007) performed 3 *in vivo* micronucleus assays with different furan exposure routes: 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). In these experiments, no increased levels of micronucleated erythrocytes were detected when a sensitive flow cytometric method was used.

Table 41: Genotoxicity of furan - *In vivo* studies in mice – Bone marrow

Test system	Test object and route of exposure	Dose	End point	Results	Comments from VKM	Ref.
Chromosomal aberration test	Male B6C3F1 (n = 10) Ip injection	0, 87.5, 175 or 350 mg/kg bw (**trial 1** harvested after 17 hours) 0, 62.5, 125,or 250 mg/kg bw (**trial 2 and 3**, harvested after 36 hours) (n = 10)	Structural chrom. ab.	Positive	Positive at highest dose (250 mg/kg bw) in trial 2 and 3, when cells are harvested after 36 hours	NTP, 1993
Micronucleous assay	Male Balb/C and male CBA mice Ip and sc injections	Balb/C: 0 – 300 mg/kg bw Ip, 0 – 275 mg/kg bw sc CBA (n = 2-3) 0 – 225 mg/kg bw ip	Structural and numerical chrom. ab.	Negative		Durling *et al*., 2007
Sister chromatid exhange	Male B6C3F1 Ip injection	0, 87.5, 175 or 350 mg/kg bw (**trial 1** harvested after 23 hours). 0, 25, 50 or 100 mg/kg bw (**trial 2** harvested after 42 hours)	Chromatid exchanges	Negative		NTP, 1993

Ref. – reference, chrom.ab. - chromosomal aberration, Ip – intraperitoneal, sc – subcutaneous

<u>*Turkey*</u>

Turkey eggs (BELT) assay

The studies commented on below are summarised in Table 42.

In recent study by Jeffrey *et al*. (2012) the DNA damaging effect of furan was studied in livers of turkey fetuses using the Comet assay. The turkey eggs assay has previously been used as an alternative to carcinogenicity/genotoxicity assays with the liver as target organ (Enzmann *et al*., 1992, 1995; Enzmann and Brunnemann, 1997; Brunnemann *et al*., 2002; Perrone *et al*., 2004; Williams *et al*., 2011a,b) and the assay is referred to as the Bird Egg Liver Toxicity (BELT) assay. It has been shown (Perrone *et al*., 2004) that turkey fetal livers on day 20 of development expressed a variety of xenobiotic biotransformation enzymes, which can activate pro-carcinogens like 2-acetylaminofluorene, aflatoxin B1, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, benzo[a]pyrene, and 4,4´-methylenebis-(2-chloroaniline), and that all compounds formed DNA adducts as measured by ^{32}P-postlabeling and DNA strand breaks as measured by the Comet assay (Williams *et al*., 2011b).

In this study by Jeffrey *et al.* (2012) a single dose of furan was injected into turkey eggs at day 23 after start of incubation of eggs at 37ºC. At this time the liver is well developed. After 24 hours of exposure to different doses (2-20 µmoles), the livers were removed from the fetuses. The "normal" alkaline Comet assay was performed on liver cells for the detection of DNA strand breaks and a modification of the Comet assay, with proteinase K, was performed for the detection of DNA strand breaks in the presence of DNA-protein crosslinks (DPXL). 1,3-Propandiol (PDO), which is metabolized to the dialdehyde, malondialdehyde (MDA), was used as a positive control for induction of DPXL. In the Comet assay without proteinase K a dose related decrease in % DNA in tail, as a measure of DNA strand breaks was observed. This effect (the negative slope) was consistently observed in 7 independent experiments, indicating that furan induced DPXL in turkey fetal liver cells. On the contrary, a positive slope was observed with proteinase K, indicative of removal of DPXL and expression of single and/or double DNA strand breaks. This study clearly indicated that furan induced DPXL and DNA single and/or double strand breaks. Effects were observed at low exposures, corresponding to 2 µmol/0.6 g liver (= 0.23 mg/g liver), assuming that the entire administrated dose was metabolised by the liver.

For comparison, in the study by Cordelli *et al*. (2010) the lowest effective dose in the Comet assay on mouse liver corresponded to 5.12 mg/g liver.

Table 42: Genotoxicity of furan in liver of turkey foetuses (Jeffrey *et al*., 2012)

Test system	Test object and route of exposure	Dose	End point	Results
Standard Comet assay on liver of foetuses	BELT assay on Turkey eggs	2-20 µ mol for 24 hours	Primary DNA damage leading to strand breaks	Negative, but with indication of DNA-DNA cross-links (dose dependant decrease in DNA damage)
Modified Comet assay for the detection of crosslinking agents on liver of fetuses	BELT assay on Turkey eggs	2-20 µ mol for 24 hours	Primary DNA damage leading to strand breaks and DNA-protein crosslinks	Positive (DNA-protein cross-links)

4.3.2.2 Summary of in vivo studies

In vivo experiments on genotoxicity of furan showed conflicting results. Furan has been tested in different species: rats, mice and turkey fetuses and different organs: liver, spleen and bone marrow/peripheral blood.

Only few studies have been published on the genotoxicity of furan in **rats** so far. Negative results were obtained for the induction of UDS in the ***liver*** at a relative high dose. However

DNA adducts were measured after exposure to low doses and also DNA damage measured by the Comet assay (Neuwirth *et al*., 2012). In another recent study in rats (McDaniel *et al*. (2012)), furan induced DNA strand breaks but not gene mutations in the liver.

VKM finds it surprising that increase in mutations was not observed in the rat liver in the *cII* assay at doses that were higher than used in the NTP cancer bioassay, with clear liver toxicity at the highest dose (30 mg/kg bw), while positive responses were observed in the Comet assay. The discrepancy between the results in these assays could be due to the repair of the primary DNA damage measured in the Comet assay or cell death (apoptosis/necrosis) of cells with DNA damage. It should be mentioned that DNA damage is only observed at high toxic doses. On the other hand, the negative results could also be due to non-optimal sampling times (time between last treatment and time of sampling). In order to save animals, the same sampling time (24 hours) was used for all genetic end points in the present study. The sampling time is the period needed for a mutation to be fixed and is a critical variable in mutation tests. A sampling time of 3 days is recommended for fast proliferating tissues (OECD 488), and an even longer sampling time may be needed for slow proliferating tissues as the liver. Therefore, the sampling time for the *cII* assay might be too short, although some accumulation of mutants could be expected during the 8 weeks treatment time. For the Comet assay the sampling time is longer than recommended (2-6 hours), and in a study by Ding *et al*. (2012) the optimal sampling time for the Comet assay performed on rats orally exposed to 16 mg/kg bw of furan was claimed to be 1 hour. It is therefore possible that at a shorter sampling time a higher response at lower doses would have been observed. Unfortunately, this very comprehensive study cannot give a clear answer to the question on the mechanism of action (MOA) of furan toxicity, although the study authors concluded that the results point to a non-genotoxic mode of action.

The data on mitogen-stimulated splenocytes in mice is supported by a similar study in rats, where low doses induced chromosomal aberrations in mitogen stimulated splenocytes (Neuwirth *et al*., 2012).

Also in rats, genotoxicity data performed on bone marrow or peripheral blood cells were consistently negative for gene mutations, CA, MN, SCE and DNA strand breaks.

In **mice** the most important studies were performed by Cordelli *et al*. (2010), and Leopardi *et al*. (2010). They used the same mouse strain, exposure route and doses as in the NTP carcinogenicity study as well as higher acute doses. At the low doses there were only indications of genotoxicity in the ***liver*** (overexpression of several DNA repair genes). Furan induced DNA strand breaks and showed indication of DNA-cross-links, measured by the Comet assay, in the liver, but at high acute doses only. No induction of DNA repair was measured in the UDS assay.

At low repeated doses furan induced micronuclei and cross-links in mitogen stimulated ***splenocytes***. DNA damage was not observed in non-stimulated splenocytes.

Several studies performed on the ***bone marrow*** with different exposure routes were consistently negative for CA, MN and SCE.

In the ***liver*** of **turkey fetuses** relatively low doses of furan induced a dose-dependent decrease in DNA strand breaks in the standard Comet assay. In a modified version, where DNA-protein crosslinks were cleaved by proteinase K, a dose-dependent increase was observed in DNA strand breaks. This study gives a clear indication that furan, or its reactive metabolite, BDA, can cause DNA-DNA and DNA-protein crosslinks.

4.3.3 Summary of genotoxic effect of furan and BDA

In *in vitro* studies conflicting results have been obtained for furan, with many negative results in standard assays. However, genotoxic effects of furan were found *in vitro*, but only when tested under appropriate test conditions, whereas its metabolite BDA was consistently genotoxic, with a much higher potency than the parent compound. In general, mainly chromosomal aberrations were induced, but gene mutations in mammalian cells and in special sensitive strains of *Salmonella typhimurium* were also observed. Some of the negative results found *in vitro* with furan might be due to: 1) volatility of furan with subsequent reduction in test media/plates concentrations, 2) the insensitivity of the assay for the specific end point 3) inadequate metabolic activation of furan to BDA.

In vitro studies with BDA, the active metabolite of furan, were positive in a narrow concentration range in nearly all studies reported. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed.

Recent *in vivo* studies of furan, where different end points of genotoxicity and the expression of DNA damage related genes were measured, show genotoxic effects in the spleen and possibly also in the liver even at low exposure levels. At high exposure levels, DNA damage was measured in the liver of mice and rats, when standard tests were performed. DNA-DNA and DNA-protein crosslinks were observed in the liver of turkey fetuses at low exposure level, when the assay was modified to detect these specific kinds of DNA damages.

Although furan produced negative results in some *in vivo* studies, many of these negative *in vivo* tests may not be the most relevant for genotoxicity testing of furan (gene mutations in blood cells, micronucleus assay in bone marrow in rodents and UDS assay in liver).

The negative results obtained for furan *in vivo* could be due to: 1) the genotoxic effects were measured in other tissues than the target organ (liver), and the reactive metabolite, BDA, did not reach these tissues, 2) a crosslinking agent like BDA is not detected in standard genotoxicity tests (e.g. the standard Comet assay) or only at high exposure level, 3) DNA damages were not detected due to non-appropriate sampling times used in some *in vivo* studies.

As supporting evidence to a genotoxic mechanism of action, for the carcinogenicity of furan, radio labelled furan has recently been reported to bind to DNA in a dose-dependent manner after oral exposure to male rats at low doses (close to the estimated human exposure dose).

Available *in vivo* data with furan indicate that a reactive metabolite, most likely BDA, is formed and that this metabolite can react with DNA and induce mutations. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed, but BDA was found genotoxic in several *in vitro* tests. VKM therefore considers that genotoxic mechanisms in furan induced carcinogenesis cannot be excluded.

4.4 Carcinogenicity

4.4.1 Mice

B6C3F1 mice (n=50/dose/gender) were given furan in corn oil by gavage at doses of 0, 8 and 15 mg/kg bw five days a week for 2 years (see also section for chronic toxicity). The incidence of hepatocellular adenomas and carcinomas both separately and in combination were significantly increased in both male and female mice in both dose groups (Table 43). NTP concluded that there is a clear evidence of carcinogenicity for both male and female mice based on the liver tumours (NTP, 1993).

Groups of female B6C3F1 mice were given furan by gavage at 0 (n = 50), 0.5 (n = 100), 1.0 (n = 75), 2.0 (n = 50), 4.0 (n = 50) and 8.0 (n = 50) mg/kg bw five days a week for 2 years. Complete gross examination and macroscopic examination of the liver was performed at necropsy. A statistical significant increase in the incidence of hepatocellular foci in mice exposed to 4.0 and 8.0 mg furan/kg bw was observed. Significant increases in the incidence of hepatocellular adenomas were also observed in the same dose groups. Hepatocellular carcinomas significantly increased in mice exposed to 8.0 mg furan/kg bw, and combined adenomas and carcinomas were significantly increased in mice exposed to 4.0 and 8.0 mg furan/kg bw (Moser *et al*., 2009).

Table 43: Incidences of tumours in mice taken from NTP (1993) and Moser *et al*., (2009)

Tumour	Females							Ref.
	Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg	15 mg/kg	
Hepatocellular adenomas	5/50 (10%)	-	-	-	-	31/50* (62%)	48/50* (96%)	NTP, 1993
Hepatocellular carcinomas	2/50 (4%)	-	-	-	-	7/50* (14%)	27/50* (54%)	NTP, 1993
Hepatocellular adenomas and carcinomas	7/50 (14%)	-	-	-	-	34/50* (68%)	50/50* (100%)	NTP, 1993
Hepatocellular adenomas	3/36 (8%)	4/72 (6%)	4/53 (8%)	4/41 (10%)	11/36* (31%)	25/39* (64%)	-	Moser *et al*., 2009
Hepatocellular carcinomas	0/36 (0%)	4/72 (6%)	4/53 (4%)	1/41 (2%)	2/36* (6%)	11/39* (28%)	-	Moser *et al*., 2009
Hepatocellular adenomas and carcinomas	3/36 (8%)	8/72 (11%)	6/53 (11%)	5/41 (12%)	12/36 (33%)	29/39 (74%)	-	Moser *et al*., 2009
Tumour	Males							**Ref.**
	Control	**0.5 mg/kg**	**1 mg/kg**	**2 mg/kg**	**4 mg/kg**	**8 mg/kg**	**15 mg/kg**	
Hepatocellular adenomas	20/50 (40%)	-	-	-	-	33/50* (66%)	42/50* (84%)	NTP, 1993
Hepatocellular carcinomas	7/50 (14%)	-	-	-	-	32/50* (64%)	34/50* (68%)	NTP, 1993
Hepatocellular adenomas and carcinomas	26/50 (52%)	-	-	-	-	44/50* (88%)	50/50* (100%)	NTP, 1993

*Significant increase as compared to control mice.
- denotes "not tested".

4.4.2 Rats

F344 rats (n=70/dose/gender) were given furan by gavage at 0, 2, 4 and 8 mg/kg bw in corn oil for 5 days a week in 2 years. After dosing for 9 and 15 months, 10 rats from each group were evaluated for the presence of treatment-associated lesions. A significant and dose-related increase in the incidence of cholangiocarcinomas was observed in male and female rats in the 9-month interim study (Table 44). In the 15-month interim study, 78% and 90% incidences of cholangiocarcinomas were observed at the lowest dose in males and females, respectively. In all dose groups in the 2-year study, cholangiocarcinomas of the liver were observed with an overall rate of 98% in female rats at all doses after 2 years (Table 44). The incidences of hepatocellular adenomas and carcinomas (both separate and combined) increased significantly

and dose-dependent in male rats after 2-years at 4 and 8 mg/kg bw. Hepatocellular adenomas increased significantly in female rats at the same dose levels. The incidence of mononuclear cell leukemia increased in male and female rats receiving 4 and 8 mg furan/kg bw, and the incidence of the 8 mg/kg bw group exceeded the historical control ranges for corn oil gavage studies. NTP concluded that there is a clear evidence of carcinogenicity in both male and female rats based on liver tumours, cholangiocarcinoma and mononuclear cell leukemia (NTP, 1993).

Table 44: Incidence of tumours in rats taken from NTP (1993).

Tumour	Females				Ref.
	Control	2 mg/kg	4 mg/kg	8 mg/kg	
Cholangiocarcinomas (9-month interim)	0/10 (0%)	4/10* (40%)	9/10* (90%)	10/10* (100%)	NTP, 1993
Cholangiocarcinomas (15-month interim)	0/9 (0%)	9/10* (90%)	9/9* (100%)	7/7* (100%)	NTP, 1993
Cholangiocarcinomas	0/50 (0%)	49/50* (98%)	50/50* (100%)	48/50* (96%)	NTP, 1993
Hepatocellular adenomas	0/50 (0%)	2/50 (4%)	4/50* (8%)	7/50* (14%)	NTP, 1993
Hepatocellular carcinomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	NTP, 1993
Hepatocellular adenomas and carcinomas	0/50 (0%)	2/50 (4%)	4/50* (8%)	8/50* (16%)	NTP, 1993
Mononuclear cell leukemia	8/50 (16%)	9/50 (18%)	17/50* (34%)	21/50* (42%)	NTP, 1993
Tumour	**Males**				**Ref.**
	Control	**2 mg/kg**	**4 mg/kg**	**8 mg/kg**	
Cholangiocarcinomas (9-month interim)	0/10 (0%)	5/10* (50%)	7/10* (70%)	10/10* (100%)	NTP, 1993
Cholangiocarcinomas (15-month interim)	0/9 (0%)	7/9* (78%)	9/9* (100%)	6/6* (100%)	NTP, 1993
Cholangiocarcinomas	0/50 (0%)	43/50* (86%)	48/50* (96%)	49/50* (98%)	NTP, 1993
Hepatocellular adenomas	1/50 (2%)	4/50 (8%)	18/50* (36%)	27/50* (54%)	NTP, 1993
Hepatocellular carcinomas	0/50 (0%)	1/50 (2%)	6/50* (12%)	18/50* (36%)	NTP, 1993
Hepatocellular adenomas and carcinomas	1/50 (2%)	5/50 (10%)	22/50* (44%)	35/50* (70%)	NTP, 1993
Mononuclear cell leukemia	8/50 (16%)	11/50 (22%)	17/50* (34%)	25/50* (50%)	NTP, 1993

*Significant increase as compared to control rats. – denotes "not tested".

4.4.3 Summary of carcinogenicity

The carcinogenicity studies show significant correlation between furan dose and increasing incidence of hepatocellular adenomas and carcinomas in male and female mice and rats. In addition, a dose-dependent increase in mononuclear cell leukemia was observed in male and female rats. The increase in cholangiocarcinomas in male and female rats was considered by VKM as the most adverse tumour development observed in rodents, with a 98% incidence already at the lowest furan dose in female rats. The background level of cholangiocarcinoma

development in this rat strain is normally low after chemical exposure. A considerable amount of furan metabolites are excreted in the bile and may consequently cause the incidence of cholangiocarcinomas in rats exposed to furan. Since the lowest furan dose tested induced nearly 100% incidence of cholangiocarcinomas in female rats, new carcinogenicity studies with testing of lower furan doses are needed for a more robust risk assessment of furan in food.

4.5 Mechanisms of action

Several mechanisms have been suggested to be involved in the toxicity and carcinogenicity of furan. The genotoxicity of furan has been described in a separate section (see section 4.3). The involvement of other mechanisms such as irreversible chronic inflammation associated with secondary oxidative DNA damage, oxidative stress, increased proliferation and reduced apoptosis will be described shortly in this section. The evidence for cholangiofibrosis as a pre-stage in the development of cholangiocarcinomas and the differences in histopathology of the cholangiocarcinomas between rats and humans will also be addressed.

The liver is the main target organ for furan toxicity. In the most sensitive species, the rat, both hepatocellular carcinomas and cholangiocarcinomas were observed in the liver. However, it has been argued that the major mechanism in the development of cholangiocarcinomas is oxidative stress, and that indirect secondary DNA damage rather than a direct genotoxic mechanism is involved (Hickling *et al*., 2010). Sprague Dawley rats (n=5/time point) were administered 30 mg/kg bw furan for 5 days a week. Animals were put to death after 8 hr; 1, 3, 7, 10, 12, and 20 days; and 1, 2 and 3 months of furan treatment. A recovery group was included at the three-month time, after one-month off dose, for assessment of recovery. Increased necrosis and reduced apoptosis were observed in hepatocytes in all lobes of the liver after both eight hours and one day, but the caudate right (target lobe) and lateral left lobe were most severely affected. From day 12, increased incidence of biliary proliferation, intestinal metaplasia and cholangiofibrosis occurred. The hepatocyte response occurred in all liver lobes, while the biliary response was confined to areas of severe initial damage. It was shown an increase in oxidative DNA damage by an increase in 8-oxo-dG after furan treatment using immunofluorescence, both in hepatocytes in the vicinity of cholangiofibrosis and in biliary cells in the same areas. These DNA damages were persistent during the recovery phase. Microarray analyses showing an increased gene expression of genes related to oxidative stress and proliferation, both during continuous furan exposure and after the recovery phase (Hickling *et al*., 2010). An increase in the expression of genes involved in proliferation, oxidative stress and DNA repair was observed in mice exposed to furan (Cordelli *et al*., 2010), supporting the findings from Hickling *et al*. (2010). In a recent paper, changes in the expression of genes related to apoptosis, cell cycle and DNA damage were observed in Sprague Dawley rats after oral treatment with 30 mg/kg bw furan five days a week for 3 months. In addition, a change in the micro-RNA (miRNA) profile and hypermethylation of genes were observed in furan-treated rats. The authors suggested that non-genotoxic mechanisms are important for furan carcinogenicity (Chen *et al*., 2012).

It has been suggested that cholangiofibrosis is a structural anomaly that precedes the development of cholangiocarcinomas. In a “stop-study”, fifty male F344 rats were given 30 mg/kg bw furan by gavage 5 days a week for 90 days. Ten treated rats were necropsied at day 90 and the remaining 40 rats were then held without further treatment. Ten of these previously treated rats were put to death at 9- and 15-months, respectively, while the rest of the animals did not survive or were sacrificed at or prior to the 21th month of the study. A total of 21 primary cholangiocarcinomas (five from the 9 month “stop–study” and 16 from the 2-year

carcinogenicity study by NTP, 1993) were transplanted into 5-10 recipients to study the malignant properties of these tumours. "Stop-study" rats sacrificed at 9- and 15-months had gross and microscopic liver changes that were similar in severity and extent to those seen at 90 days. However, the liver changes were more severe than those seen in the rats continuously dosed with 8 mg/kg bw furan for 9- and 15-months. A 100% incidence of cholangiocarcinomas was observed already after 9-month duration of the "stop study". Cholangiocarcinomas that metastasized to pancreas, lymph nodes and spleen were observed in two 15-month rats. Four of the transplanted cholangiocarcinomas showed growth in the recipients and metastases occurred for 2 of the 4 transplantable cholangiocarcinomas. The authors conclude that the proliferative cholangial lesions are considered to be malignant or potentially malignant (study described partly in Maronpot *et al*., 1991 and NTP, 1993).

It has been suggested that there can be a tendency to overdiagnose the cholangiofibrosis and adenofibrosis as cholangiocarcinoma in rats. However, this has only been reported in a textbook without further references to detailed descriptions (Greaves, 2007). However, similar histopathology (intestinal differentiation) of the cholangiofibrosis and cholangiocarcinomas has also been reported by Elmore and Sirica (1993), indicating that there might be some difficulties in separating the cholangiocarcinomas from the cholangiofibrotic lesions. Elmore and Sirica (1993) studied the histopathology of the furan-induced cholangiocarcinomas (also called primary hepatic adenocarcinomas by the author) in rats and found that 96% of the cholangiocarcinomas were characterized by intestinal cell differentiation (i.e. goblet cells, Paneth cells and neuroendocrine cells). The author had previously shown similar changes in cell structure in cholangiofibrotic lesions induced in rat liver after 2-4 weeks of furan treatment. This strongly suggest that cholangiofibrosis is connected to the later development of cholangiocarcinomas. In cases where a benign tumour is known to be able to progress to a malign tumour, e.g. hepatocellular adenomas to carcinomas, they are often considered together in the risk characterisation. Thus, the possibility of over-diagnosis of cholangiocarcinomas due to the presence of cholangiofibrosis will most likely not affect the risk characterisation. This is the case in the present situation where cholangiocarcinomas were found in nearly 100% at the low dose already after 15 months. Small intestinal metaplasia has been suggested as a risk factor in the development of cholangiocarcinomas in the biliary tract of humans (Elmore and Sirica, 1993).

It is expected that children can be more susceptible to genotoxic carcinogens than adults, and this has been previously characterised by U.S. Environmental Protection Agency (EPA, 2005) and Hattis *et al*. (2005). Some aspects potentially leading to childhood susceptibility include more frequent cell division, reduced level of DNA repair enzymes in some tissues and a less developed immune system (EPA, 2005). Quantification analyses based on some genotoxic carcinogens support the conclusion that there can be greater susceptibility for the development of tumours as a result of exposure to chemicals acting through a mutagenic mode of action, if the exposure occurs in early life stage as compared with later life stages. However, the overall database as basis for the analyses is of limited size (EPA, 2005; Hattis *et al*. 2005). *In vivo* studies starting furan exposure when the animals are 6-7 weeks old, do not take the increased susceptibility of children into account.

4.5.1 Summary of mechanism of action

Based on the results from the abovementioned studies, VKM finds it likely that cellular mechanisms such as oxidative stress, changes in cell cycle, proliferation and apoptosis are involved in furan-induced carcinogenesis. However, this does not exclude a concurrent genotoxic mechanism of furan (see section 4.3). Although there might be a possibility for

over-diagnosis of the cholangiocarcinomas due to the presence of cholangiofibrosis, this will most likely not affect the risk characterisation since cholangiofibrosis is known to be able to progress to carcinomas. VKM recognises that the cholangiocarcinomas from rats have the ability to give metastases when transplanted in recipient rats. This has been characterised by intestinal cell differentiation, which has been suggested as a risk factor for the development of cholangiocarcinomas in the biliary tract of humans. Therefore, VKM will take the furan-induced development of cholangiocarcinomas into account in this risk assessment of furan.

4.6 Conclusions on hazard

The liver is the main target organ for furan toxicity both in mice and rats, but the rat is the most sensitive species. A dose-dependent increase in hepatocellular adenomas and carcinomas was observed in mice and rats, and an increase in the incidence of cholangiocarcinomas was observed in rat liver. Cholangiocarcinomas in male and female rats is the most sensitive toxicological end point observed in rodents. On the basis of the available data, VKM considers that rat cholangiocarcinomas may be relevant for assessing human risk from furan.

Available *in vivo* data with furan indicate that a reactive metabolite, most likely BDA, is formed and that this metabolite can react with DNA and induce mutations. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed, but BDA was found genotoxic in several *in vitro* tests. VKM therefore considers that genotoxic mechanisms in furan-induced carcinogenesis cannot be excluded. Since a threshold cannot be identified, the substance was assessed as a genotoxic carcinogen.

5 Critical effect and choice of point of departure (POD)

In the studies on furan carcinogenesis, rats were found to be more sensitive than mice. Therefore, rats were used to establish a point of departure (POD) for furan toxicity. The calculation of a benchmark dose (BMD) and using the benchmark dose lower bound (BMDL) as a point of departure is the most appropriate method for risk characterisation of substances in food that are genotoxic and carcinogenic. In cases where data are unsuitable for deriving a benchmark dose, the use of T25, representing the dose corresponding to a 25% incidence of tumours, is recommended (EFSA, 2005). Even though the data available were not optimal, VKM considered them suitable for a BMDL approach. However, for comparison, the calculation of T25, which resulted in a value of 0.14, is described in Annex III. The Panels have chosen to base the risk assessment of furan on a POD derived using the BMD method as described below.

5.1 Summary of previous BMD calculations for furan in food

An expert group established by the International Life Science Institute – European branch (ILSI Europe) calculated the BMD and used the BMDL as a point of departure in the risk assessment of twelve genotoxic and carcinogenic chemicals including furan (Benford *et al*., 2010). The risk assessment on furan is described in more detail in the paper by Carthew and co-workers (Carthew *et al*., 2010). BMD and $BMDL_{(1,\ 5\ and\ 10)}$ were calculated for the main toxicological effects in mice and rats: cholangiocarcinomas, liver adenomas and carcinomas and mononuclear cell leukaemia. The $BMDL_{10}$ were used for further risk assessment, and the lowest calculated $BMDL_{10}$ (0.0012 mg/kg bw/day in the text, but 0.000723 in the table) were found for the male rat cholangiocarcinomas. The difference between the BMD and $BMDL_{10}$

for cholangiocarcinomas was more than 70-fold, indicating high confidence interval and large uncertainties in the BMD calculations. This is most likely caused by a lack of dose-response in the development of cholangiocarcinomas, which shows 96% incidence at the lowest furan dose of 2 mg/kg bw/day in female rats. The authors in Carthew *et al*. (2010) argued that the cholangiocarcinomas induced by furan in rat are most likely derived by a mechanism involving oxidative stress and indirect DNA damage rather than a direct genotoxic mechanism (Hickling *et al*., 2010). In this paper, an increase in oxidative DNA damage by an increase in 8-oxo-dG after furan treatment using immunofluorescence, and microarray analyses showing an increased expression of genes related to oxidative stress, were shown. Carthew and co-workers suggested that a genotoxic mechanism was more likely for the development of hepatocellular adenomas and carcinomas than for cholangiocarcinomas. Therefore, the experts disregarded the $BMDL_{10}$ for the cholangiocarcinomas, choosing the lowest $BMDL_{10}$ based on hepatocellular adenomas and carcinomas in male rats of 1.28 mg/kg bw/day based on the model average to derive MOE.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently published a risk assessment of furan (JECFA, 2011), where they used the BMD approach and BMDL as the point of departure. JECFA also disregarded the cholangiocarcinomas in rats for calculation of BMDL, because these neoplasms were only observed in rats and were associated with extreme hepatotoxicity. In addition, these neoplasms were, according to JECFA, also associated with early and marked biliary tract proliferative response. Furthermore, JECFA argued that the relevance for humans of the cholangiocarcinomas was not clear, and the high incidence of these neoplasms at all furan doses precluded identification of a POD. The Committee chose to use the $BMDL_{10}$ of 1.34 mg/kg bw calculated from the development of hepatocellular adenomas and carcinomas in female mice from the Moser *et al*. (2009) study as the POD. The $BMDL_{10}$ corresponds to 0.96 mg/kg bw/day when adjusted from a 5 days/week dosing schedule to an average daily dose.

5.2 Selection of POD from previous risk assessments of furan

The most critical toxicological effect observed at the lowest furan dose (2 mg/kg bw/day) is the development of cholangiocarcinomas in male and female rats. Both the expert groups of ILSI Europe and JECFA (Carthew *et al*., 2010; JECFA, 2011) have disregarded the cholangiocarcinomas as the basis for the BMDL calculations. It is the view of VKM that several mechanisms, such as oxidative stress and proliferation are likely to be involved in the development of cholangiocarcinomas, but this does not exclude the possible involvement of a genotoxic mechanism. The relevance of cholangiocarcinomas for humans can also not be excluded, since this type of tumours does occur in humans, often with severe outcome as described by Hickling *et al*., (2010). However, the histopathology of the cholangiocarcinomas in rats is described as less malignant than its counterpart in humans. It is also suggested that cholangiofibrosis is connected to the later development of cholangiocarcinomas, due to similarities in histopathology. Small intestinal metaplasia as observed in both cholangiofibrosis and cholangiocarcinomas in rats has been suggested as a risk factor in the development of cholangiocarcinomas in the biliary tract of humans (Elmore and Sirica, 1993). VKM agrees with JECFA that the data from the NTP study (NTP, 1993) on the development of cholangiocarcinomas after 2 year are not suitable for calculating a BMDL as a POD for the toxicological risk assessment of furan, since a dose-response for this toxic effect is lacking. This is clearly shown by the large difference in BMD and the corresponding BMDL for the development of the cholangiocarcinomas as calculated by the ILSI expert group. However, the calculated BMDLs by ILSI Europe (1.28 mg/kg bw/day) and JECFA (0.96 mg/kg bw/day) based on hepatocellular adenomas and carcinomas in rats and mice, respectively, are close to

the lowest reported effect level of 1.42 mg/kg bw/day in female rats (after recalculation of the dose to a daily exposure) at which a 98% increase in incidence of cholangiocarcinomas was observed.

5.3 Calculation of BMD with PROAST software and determination of POD

For the present risk assessment, BMD calculations for the cholangiofibrosis and cholangiocarcinomas for all time points (9- and 15-months interim and 2 year) from the NTP study were calculated. Since there were no difference between the sexes, BMD calculations for the cholangiocarcinomas for all time points were combined with time as a covariate. The BMD for the cholangiocarcinomas from the 15-month interim study, the 2-year study and the combined calculation were all rejected due to no acceptable fitted curves and high confidence intervals caused by the lack of a dose-response, indicating large uncertainty in the calculations.

The incidence of cholangiocarcinomas in rats from the 9-month interim evaluation of the 2-year study by NTP was used to calculate a BMD and BMDL for furan as a POD for carcinogenicity. Although these data is based on ten animals only and is derived from a sub-chronic time point, these are data from the most critical carcinogenic effect observed in rats. Initially, the BMD calculations were performed in PROAST, with sex as covariate, increasing the group size. However, the calculations showed that there were no difference between the sexes, and therefore the data for females and males could be combined, resulting in one overall BMDL (Table 45).

Table 45: BMD and BMDL calculations on cholangiocarcinomas from the 9-month interim evaluation of NTP (1993), combining the sexes.

End point	Model	Number of parameters	Loglik	Accept	BMD_{10}	$BMDL_{10}$	$BMDU_{10}$
Cholangio-carcinomas (9-month interim, sex combined)	Zero	1	-54.83	-	NA	NA	NA
	Full	8	-23.02	-	NA	NA	NA
	One-stage	2	-25.01	yes	0.27	0.20	0.36
	Two-stage	3	-25.01	yes	0.27	0.20	0.36
	Log-logistic	3	-24.43	yes	1.04	0.46	1.5
	Weibull	3	-23.92	yes	0.71	0.22	1.28
	LogProbit	3	-24.2	yes	1.05	0.47	1.52
	Gamma	3	-24.01	yes	0.83	0.33	1.42
	Logistic	2	-25.4	yes	1.01	0.68	1.45
	Probit	2	-25.1	yes	0.70	NA	NA
	LVM-E2	2	-25.1	yes	0.97	0.66	1.43
	LVM-H2	2	-25.57	yes	0.49	0.35	0.66

Accept. – Acceptability

All models showed acceptable fit according to the PROAST program (see Appendix II, Table A3 and Figure A1 for more details on the BMD calculations). The calculated BMDLs for the accepted models differ with less than one order of magnitude, and all have acceptable confidence intervals (i.e. ratio $BMDL_{10}/BMDU_{10} < 10$). The lowest $BMDL_{10}$ of 0.2 mg/kg bw was calculated for the one-stage and two-stage models. This $BMDL_{10}$ value has been re-calculated from 5-days exposure per week to a daily exposure, giving a $BMDL_{10}$ of 0.14 mg/kg bw, and has been included in Table 46 below.

An overview over the reported and calculated PODs for carcinogenicity of furan is given in Table 46.

Table 46: Reported and calculated PODs for furan carcinogenicity.

Critical effect	Point of departure (POD) mg/kg bw/day	Method	Study	Reference
Cholangiocarcinomas in rats	1.42	LOAEL	2-year carcinogenicity study (NTP)	NTP, 1993
Hepatocellular adenomas and carcinomas in rats	1.28	$BMDL_{10}$	2-year carcinogenicity study (NTP)	Carthew *et al.*, 2010
Hepatocellular adenomas and carcinomas in female mice	0.96	$BMDL_{10}$	2-year carcinogenicity study (Moser *et al.*, 2009)	JECFA, 2011
Cholangiocarcinomas in rats (sex combined)	0.14	$BMDL_{10}$	9-month interim evaluation from 2-year carcinogenicity study (NTP)	VKM, 2012

The lowest calculated or reported POD for furan from Table 46 is the $BMDL_{10}$ of 0.14 mg/kg bw/day from the cholangiocarcinomas in rats (sex combined) from the 9-month interim evaluation. This $BMDL_{10}$ will be used in the further risk assessment of furan.

5.3.1 Correction factor

The data on the development of cholangiocarcinomas in rats after 2 year of the NTP study (NTP, 1993) were not suitable for calculating a BMDL as a POD for furan toxicity, since there was close to 100% incidence at the lowest dose and therefore a dose-response for this toxic effect was lacking. As a sub-optimal solution VKM used the cholangiocarcinomas from the 9-month interim study (sex combined) to derive a POD, because these data showed a dose-response and were considered suitable for a BMDL calculation. Since the time point (9-month) used in deriving the BMDL was shorter than a full life span (2-year) study of the rat, a correction factor had to be included to adjust for shorter exposure and observation time (Dybing *et al.*, 1997; ECHA, 2010). The 9-month interimstudy represents a 2.66 times (24 months/9months=2.66) shorter duration of exposure and observation compared to the full length study of 24 months. The combined correction factor will be 7, when taking into account both shorter exposure time and reduced observation time (2.66 x 2.66 = 7).

5.3.2 Determination of POD

Applying a correction factor of 7 the $BMDL_{10}$ of 0.14 mg/kg bw/day gave a POD of 0.02 mg furan/kg bw/day. This POD of 0.02 mg/kg bw/day, which was used in the risk characterisation, represents the most conservative POD based on the development of cholangiocarcinomas from the 9-month interim study, and would probably be an overestimation of the hazard. It is noted that the tumour incidence increased by about 2.5 to close to 100% from 9 to 24 months (Table 44).

6 Risk characterisation

In rodents, the liver is the main target organ for furan toxicity (see sections on general toxicity and carcinogenicity). Carcinogenicity studies with oral furan exposure have revealed significant dose-dependent increase in the combined incidence of hepatocellular adenomas and carcinomas in male and female B6C3F1 mice (NTP, 1993; Moser *et al*., 2009) and in male F344/N rats (NTP, 1993). Moreover, high incidences (approximately 86-100%) of cholangiocarcinomas were reported in F344/N rats of both sexes after 2 years of oral exposure, even at the lowest furan dose tested (NTP, 1993). In addition, an increased incidence of mononuclear cell leukaemia was observed in male and female rats at the two top doses, where the incidence at the top dose exceeded the historical control ranges. Furthermore, liver non-neoplastic lesion incidences were abundant in both B6C3F1 mice and F344/N rats at all tested furan doses in the 2-year experiments (NTP, 1993; Moser *et al*., 2009). In the 90-day study in rats by Gill *et al*. (2010), biliary tract hyperplasia and cholangiofibrosis were observed which are related to the development of cholangiocarcinomas in rats reported by NTP (NTP, 1993).

Available *in vivo* data with furan indicate that a reactive metabolite, most likely BDA, is formed and that this metabolite can react with DNA and induce mutations. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed, but BDA was found genotoxic in several *in vitro* tests. VKM therefore considers that genotoxic mechanisms in furan-induced carcinogenesis cannot be excluded. Since a threshold cannot be identified, the substance was assessed as a genotoxic carcinogen.

The development of cholangiocarcinomas (sex combined) from the 9-month interim study of NTP (1993) was used to calculate a POD of 0.02 mg/kg bw/day, which has been chosen in the risk characterisation for calculating margin of exposure (MOE) for the different age groups. The MOE is the ratio between the calculated POD from the critical toxicity in animal studies and the calculated human intake. A MOE will be calculated for the most important exposure data for all age groups. The lower the MOE is, the higher the possible risk for negative health effects will be. EFSA concluded that for a genotoxic compound, a guidance MOE of 10 000 or higher if it is based on the $BMDL_{10}$ from an animal study would be of low concern from a public health point of view (EFSA, 2005). However, the risk assessors should take into account the quality of the hazard characterisation and intake data and the uncertainties inherent in the data used in the interpretation of the calculated MOE. It should also be noted that in this guidance MOE, safety factors are included.

6.1 Margin of exposure calculations for 6-month-old infants

The exposure calculations for 6-month-old infants show that the major contribution to the furan exposure comes from jarred baby food (Table 11). When the furan exposure was calculated for the consumers of jarred baby food, the mean and high (95 percentile) furan

exposure was 0.18 µg/kg bw/day and 0.45 µg/kg bw/day, respectively. This will give MOEs of 111 and 44 for the two respective exposure calculations (Table 47). For 6-month-old consumers of jarred baby food, with an additional contribution from infant formula, milk products and commercial powder-based porridge, the respective mean and high furan exposures were calculated to 0.26 and 0.58 µg/kg bw/day, with corresponding MOE-values of 77 and 34 (Table 47).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 6-month-old infants. The mean and high furan exposures among all participants were calculated to 0.17 and 0.51 µg/kg bw/day, giving respective MOE-values of 118 and 39 (Table 47).

The calculated furan exposure among the consumers of jarred baby food with an additional contribution from all relevant food categories is not far from the population average in 6-month-olds (see section 3.1.11).

The calculated mean and high (95 percentile) furan exposures among 6 month-old non-breastfed consumers of jarred baby food and other relevant food categories were found to be 0.35 and 0.65 µg/kg bw/day (Table 12), with corresponding MOEs of 400 and 215, respectively. These MOEs for non-breastfed infants are in the same range as for consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge among all participants (Table 47).

Table 47: Margin of exposure (MOE) for furan in 6-month-old infants for consumers of jarred baby food (n=1220), for consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge (n=1220) and for average furan exposure in all participants (n=1986) (see Table 11).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of jarred baby food	0.18	0.45	111	44
Consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial porridge	0.26	0.58	77	34
Average furan exposure from jarred baby food, milk products, infant formula and commercial powder-based porridge in all participants	0.17	0.51	118	39

6.2 Margin of exposure calculations for 12-month-old infants

The exposure calculations for 12-month-old infants show that the major contribution to the furan exposure comes from jarred baby food (Table 17). When the furan exposure was calculated for consumers of jarred baby food, the mean and high (95 percentile) furan exposures were 0.56 µg/kg bw/day and 1.30 µg/kg bw/day, respectively. This will give MOEs of 36 and 15 for the two respective exposure calculations (Table 48). For 12-month-old consumers of jarred baby food, with an additional contribution from infant formula, milk

products and commercial powder-based porridge, the respective mean and high furan exposures were calculated to 0.69 and 1.51 µg/kg bw/day, with corresponding MOEs of 29 and 13 (Table 48).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 12-month-old infants. The mean and high furan exposures among all participants were calculated to 0.62 and 1.43 µg/kg bw/day, giving respective MOE-values of 32 and 14 (Table 48).

The calculated furan exposure among the consumers of jarred baby food with an additional contribution from all relevant food categories is not far from the population average in 12-month-olds (see section 3.1.11).

The calculated furan exposure among 12-month-old non-breastfed infants (Table 18) did not differ considerably from the abovementioned results, and are therefore covered by the calculated MOEs (Table 48).

Table 48: Margin of exposure (MOE) for furan in 12-month-old infants for consumers of jarred baby food (n=1436), for consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge (n=1436) and for average furan exposure in all participants (n=1635) (see Table 17).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of jarred baby food	0.56	1.30	36	15
Consumers of jarred baby food with an additional contribution from infant formula, milk products and commercial powder-based porridge	0.69	1.51	29	13
Average furan exposure from jarred baby food, milk products, infant formula and commercial powder-based porridge in all participants	0.62	1.43	32	14

6.3 Margin of exposure calculations for 24-month-old children

The exposure calculations for 24-month-old children show that the major contribution to the furan exposure comes from jarred baby food (Table 21). When the furan exposure was calculated for consumers of jarred baby food, the mean and high (95 percentile) furan exposures were 0.18 µg/kg bw/day and 0.64 µg/kg bw/day, respectively. This will give MOEs of 111 and 31 for the two respective exposure calculations (Table 49). For 24-month-old consumers of jarred baby food, with an additional contribution from other relevant food categories, the respective mean and high furan exposures were calculated to 0.25 and 0.77 µg/kg bw/day, with corresponding MOEs of 80 and 26 (Table 49).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 24-month-old children. The mean

and high furan exposures among all participants were calculated to 0.10 and 0.41 µg/kg bw/day, giving respective MOE-values of 200 and 49 (Table 49).

The calculated furan exposure among the consumers of jarred baby food with an additional contribution from all relevant food categories was found to be higher than the population average in 24-month-old children (see section 3.1.11).

Table 49: Margin of exposure (MOE) for furan in 24-month-old children for consumers of jarred baby food (n=420), for consumers of jarred baby food with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge (n=420) and for average furan exposure in all participants (n=1674) (see Table 21).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of jarred baby food	0.18	0.64	111	31
Consumers of jarred baby food with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge	0.25	0.77	80	26
Average furan exposure from jarred baby food, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and commercial powder-based porridge in all participants	0.10	0.41	200	49

6.4 Margin of exposure calculations for 4-year-old children

The exposure calculations for 4-year-old children show that the major contribution to the furan exposure comes from breakfast cereals (Table 23). When the furan exposure was calculated for 4-year-olds consumers of breakfast cereals, the mean and high (95 percentile) furan exposures were 0.03 and 0.11 µg/kg bw/day, respectively. This will give MOEs of 667 and 182 for the two respective exposure calculations (Table 50). For 4-year-old children that consume breakfast cereals, with an additional contribution from other relevant food categories, the respective mean and high furan exposures were calculated to 0.08 and 0.26 µg/kg bw/day, with corresponding MOEs of 250 and 77 (Table 50).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 4-year-old children. The respective mean and high furan exposures among all participants were calculated to 0.07 and 0.25 µg/kg bw/day, giving MOEs of 286 and 80 (Table 50).

The calculated furan exposure among the consumers of breakfast cereals with an additional contribution from all relevant food categories is in the same order of magnitude as the population average in 4-year-olds (see section 3.1.11).

Table 50: Margin of exposure (MOE) for furan in 4-year-old children for consumers of breakfast cereals (n=182), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=182) and for average furan exposure in all participants (n=391) (see Table 23).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of breakfast cereals	0.03	0.11	667	182
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks	0.08	0.26	250	77
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks in all participants	0.07	0.25	286	80

6.5 Margin of exposure calculations for 9-year-old children

The exposure calculations for 9-year-old children show that the major contribution to the furan exposure comes from breakfast cereals (Table 25). When the furan exposure was calculated for 9-year-old consumers of breakfast cereals, the mean and high (95 percentile) furan exposures were calculated to 0.02 and 0.09 µg/kg bw/day, respectively. This will give MOEs of 1000 and 222 for the two respective exposure calculations (Table 51). For 9-year-old consumers of breakfast cereals with an additional contribution from other relevant food categories, the respective mean and high furan exposures were calculated to 0.06 and 0.17 µg/kg bw/day, with corresponding MOEs of 333 and 118 (Table 51).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 9-year-old children. The mean and high furan exposures among all participants were calculated to 0.06 and 0.20 µg/kg bw/day, giving respective MOE-values of 333 and 100 (Table 51).

The calculations show that the furan exposure among the consumers of breakfast cereals with an additional contribution from all relevant food categories and the population average in 9-year-old children is in the same range (see section 3.1.11).

Table 51: Margin of exposure (MOE) for furan in 9-year-old children for consumers of breakfast cereals (n=323), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=323) and for average furan exposure in all participants (n=810) (see Table 25).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of breakfast cereals	0.02	0.09	1000	222
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks	0.06	0.17	333	118
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks in all participants	0.06	0.20	333	100

6.6 Margin of exposure calculations for 13-year-old adolescents

The exposure calculations for 13-year-old adolescents show that the major contribution to the furan exposure comes from breakfast cereals (Table 27). When the furan exposure was calculated for 13-year-old consumers of breakfast cereals, the mean and high (95 percentile) furan exposures were calculated to 0.01 and 0.05 µg/kg bw/day, respectively. This will give MOEs of 2000 and 400 for the two respective exposure calculations (Table 52). For 13-year-old consumers of breakfast cereals with an additional contribution from other relevant food categories, the respective mean and high furan exposures were calculated to 0.05 and 0.16 µg/kg bw/day, with corresponding MOEs of 400 and 125 (Table 52).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in 13-year-old adolescents. The mean and high furan exposures among all participants were calculated to 0.04 and 0.14 µg/kg bw/day, giving respective MOE-values of 500 and 143 (Table 52).

The calculations show that the furan exposure among the consumers of breakfast cereals with an additional contribution from all relevant food categories is not considerably different from the population average in 13-year-old adolescents (see section 3.1.11).

Table 52: Margin of exposure (MOE) for furan in 13-year-old adolescents for consumers of breakfast cereals (n=285), for consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, and snacks (n=285) and for average furan exposure in all participants (n=1005) (see Table 27).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of breakfast cereals only	0.01	0.05	2000	400
Consumers of breakfast cereals with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, snacks, and coffee	0.05	0.16	400	125
Average furan exposure from breakfast cereals, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, sweet breakfast cereals, chocolate and sweets, snacks, and coffee in all participants	0.04	0.14	500	143

6.7 Margin of exposure calculations for adults aged 18-70 years

The exposure calculations for adults aged 18-70 years show that the major contribution to the furan exposure comes from coffee (coffee roasted ground) (Table 29). When the furan exposure was calculated for adults that drink coffee (coffee roasted ground), the mean and high furan exposure were calculated to 0.33 µg/kg bw/day and 0.81 µg/kg bw/day, respectively. This will give MOEs of 61 and 25 for the two respective exposure calculations (Table 53). For adults that drink coffee (coffee roasted ground) with an additional contribution from consumption of other relevant food categories, the respective mean and high furan exposures were calculated to 0.34 and 0.82 µg/kg bw/day, with corresponding MOEs of 59 and 24 (Table 53).

Furan consumption from all relevant food categories among all participants, gives a population average for the mean and high furan exposure in adults aged 18-70 years. The mean and high furan exposures in all participants were calculated to 0.27 and 0.77 µg/kg bw/day, giving respective MOE-values of 74 and 26 (Table 53).

The calculated furan exposure for adults shows that the major furan exposure comes from coffee (coffee roasted ground and instant coffee) and that the contribution from other food categories is limited. The calculations show that the furan exposure among the consumers of coffee roasted ground with an additional contribution from all relevant food categories is in the same range as the population average in adults (see section 3.1.11).

Table 53: Margin of exposure (MOE) for furan in adults aged 18-70 years for consumers of coffee roasted ground (n=1398), for consumers of coffee roasted ground with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks and instant coffee (n=1398) and for average furan exposure in all participants (n=1787) (see Table 29).

	Mean furan exposure µg/kg bw/day	High furan exposure (95 percentile) µg/kg bw/day	MOE Mean exposure	MOE High exposure
Consumers of coffee roasted ground	0.33	0.81	61	25
Consumers of coffee roasted ground with an additional contribution from canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee	0.34	0.82	59	24
Average furan exposure from coffee roasted ground, canned and jarred vegetables, canned fruits, milk products, jarred tomato sauces, all breakfast cereals, chocolate and sweets, snacks, and instant coffee in all participants	0.27	0.77	74	26

6.8 Comments to the MOE

For 6-, 12- and 24-month-old children, the main source of furan exposure is jarred baby food. For 4-, 9- and 13-year-old children, the major food source to the furan exposure is breakfast cereals. In adults, the major contributor to furan exposure is coffee. The highest furan exposure was calculated for 12-month-old infants and ranged from 0.62 to 1.51 µg/kg bw/day. In adults the furan exposure ranged from 0.27 to 0.82 µg/kg bw/day.

For mean exposure among infants, children and adolescents, the MOE-values ranged from 29 in 12-month-infants to 2000 in the 13-year-old adolescents. Among high consumers, the MOE-values ranged from 13 to 400. In adults, the corresponding MOE-values ranged from 59 to 74 for mean exposure and from 24 to 26 for high exposure.

VKM considers that the current exposure to furan in all age groups, particularly among infants and children, is of health concern.

7 Uncertainty

7.1 Furan contents in food

The limit of detection (LOD) and limit of quantification (LOQ) reported for all matrixes tested were 2 and 5 µg/kg respectively. The measurement uncertainty at furan concentrations lower than 20 µg/kg was 80%, while at concentrations higher than 20 µg/kg the measurement uncertainty was 50%. Considering the given uncertainties in the analyses, the furan concentrations reported in the national monitoring data are presented with too many digits. However, VKM has chosen to use the furan concentrations as they were reported by the laboratory to ease the tracing of the results used in this opinion back to the original data source.

Mean middle bound furan concentrations from the Norwegian monitoring data were used in the exposure calculations in this opinion. Mean middle bound furan values were also used for the two different breakfast cereals and the food category snacks and crisps from the DTU report, while all furan values used from the EFSA furan database were based on the means of upper bound values. The approach of using mean upper bound values will most likely lead to an overestimate of the exposure, while using mean lower bound most likely results in an underestimate of the exposure. VKM therefore decided to use the mean middle bound values for the national monitoring data, to obtain the most realistic estimate of the exposure.

The Norwegian monitoring data present both furan data for foods analysed as purchased and for furan concentrations in foods after heat treatment (Tables 1 and 3-5). Furan contents in identical food products analysed as purchased and after heat-treatment are of the same order of magnitude, except for a few products. Only furan contents of food categories analysed as purchased were used in the calculations in this opinion.

There is lack of data on furan contents for many foods. The food categories used for furan calculations were based on results for relevant foods and food groups with high furan content previously reported in the literature. Thus, the present calculations of furan exposures in the various age groups may be underestimated.

In the literature, different furan contents for approximately the same types of foods are reported. Coffee is a major contributor to furan exposure in Norwegian adults. However, Norwegian furan analyses for coffee were not available and therefore furan values from the EFSA database were used to calculate the furan exposure from consumption of coffee. This may not be representative for Norwegian blends and preparation methods for coffee. It is not known if this fact would lead to an overestimation or an underestimation at present.

7.2 Dietary exposure assessment

Every dietary assessment is connected with uncertainty. A description of the most important uncertainties and assumptions in the dietary exposure calculations is described below.

Three concepts are fundamental to understanding the limitations of dietary assessment: habitual consumption, validity and precision (Livingstone and Black, 2003).

The habitual consumption of an individual is the person's consumption averaged over a prolonged period of time, such as weeks and months rather than days. However, this is a largely hypothetical concept; the consumption period covered in a dietary assessment is a compromise between desired goal and feasibility. In the Norwegian dietary surveys the time period covered are 14-days among the 1- and 2-year olds (Sped- and Småbarnskost 2006/2007), four consecutive days among the 4-, 9- and 13-year-olds (UNGKOST 2000) and 2 none-consecutive days among the adults (Norkost 3). Furan has been analysed and found in a relatively limited number of foods, and it is only the reported furan contents in the limited number of food groups/food items that are included in the exposure calculations. The food category with the highest furan content relevant for 4-, 9- and 13-year-olds was sweet breakfast cereals. The furan exposure could be overestimated for sweet breakfast cereals since there were only few samples and these had considerable higher furan contents than comparable food items in the EFSA database (EFSA, 2011). However, the high furan analyses reported in the DTU report were chosen rather than furan values from the EFSA database as cereal products in the Norwegian market were assumed to be more similar to the products in the Danish market. Both large within person and between person variations in consumption of furan-containing foods were seen in the consumption surveys. A large

number of repeated measurements and a comprehensive database on furan contents in different foods would be required to obtain an accurate estimate of individual furan exposure (Willett, 1998).

In this risk assessment we report furan exposure in all participants within an age group as well as the exposure in consumers only with regard to food items contributing substantially to the overall exposure, e.g. jarred baby food in children and coffee in adults. When evaluating high consumers, the uncertainty associated with the 95 percentile is higher than for the mean value, especially among the age groups with a low number of participants. However, the overall finding in this report of small differences in the 95 percentile exposure between all participants and consumers only (Tables 30 and 31), indicates that the "worst case" exposure calculations are robust.

The validity of a dietary assessment method refers to the degree to which the method actually measures the aspect of diet that it was designed to measure (Nelson and Margetts, 1997). Lack of validity is strongly associated with systematic errors (Burema *et al*., 1988). With systematic errors all respondents in a dietary study or each subgroup in a population produce the same type of error, like systematic under- or overestimation of intake. All the three different dietary assessment methods used in this risk assessment have limitations when it comes to validity. Results from validation studies among 9- and 13-year-olds indicate an underestimation of energy intake around 20% when the precoded food diary, used in UNGKOST 2000, is compared with energy expenditure (Andersen *et al*., 2005; Lillegaard and Andersen, 2005). The validation studies among 1- and 2-year-olds were performed on a previously established questionnaire, but the results showed a significantly higher energy intake with the FFQ than with the weighed record reference method (Andersen *et al*., 2003; Andersen *et al*., 2004; Andersen *et al*., 2009). The Norwegian 24-hour recall method used among adults in Norkost 3 has not been validated. However, other similar 24-hour recall methods have been validated and show an underestimation in energy intake of around 15% (Subar *et al*., 2003; Poslusna *et al*., 2009). Underestimation of energy intake indicates that not all foods eaten are reported, but not which foods are underreported. It has been shown that foods perceived as unhealthy such as fats, sweets, desserts and snacks tend to be underreported to a larger degree than foods perceived as healthy (Olafsdottir *et al*., 2006). However, among children and adolescents there have been studies were this selective underreporting was not shown (Sjøberg *et al*., 2003; Lillegaard and Andersen, 2005). As furan is found in foods perceived both as unhealthy and healthy, it is not likely that the misreporting would strongly bias the estimated furan exposure. However, if underreporting of furan containing foods is of the same magnitude as for total energy, the estimates for furan exposure are more likely to be under- than over reported.

The precision of a technique is one that gives the same answer on repeated administrations (Livingstone and Black, 2003). Poor precision derives from large random errors in the techniques of dietary assessment. The effect of random errors can be reduced by increasing the number of observations, but cannot be entirely eliminated (Rothman, 2002).

The questionnaire used among the 6-month-old children does not ask for portion sizes for jarred baby food, only for frequencies. To be able to calculate a scenario for furan the portion size was set to ¼ a jar (50 g). The jar sizes differ between different baby foods and different labels, but in these scenarios the jar size depicted in the photographic booklet for the 1- and 2-year-old children has been used. It is likely that the 6-month-olds do not eat large portions of jarred baby food, since they are in a process of adaptation to solid foods.

The questionnaires used in Spedkost 2006-2007 do not separate between commercial porridge with or without fruits. In the estimates presented in Table 11 and 12, a conservative approach

has been chosen to assume that all commercial porridge consumed is porridge with fruits. Porridge with fruits has been found to contain more than three times the amount of furan compared with porridge without fruit.

The data collection in UNGKOST 2000 was performed in year 2000-2001, and dietary patterns are constantly changing. Especially among some groups of adolescents, coffee drinks seem to be more popular now than what the 12-year-old data suggests.

It is unclear to which extent a low participation rate will influence the assessment of furan exposure. It has been shown that health conscious people are more likely to participate in a dietary survey. This can indicate a somewhat different dietary pattern among the participants than among the whole population. The direction of the uncertainty is difficult to estimate.

The dietary exposure to furan in this opinion has been calculated relative to body weight (µg/kg bw/day) for all age groups. The individual consumption data taken from the dietary surveys should ideally be paired with data on body weights for the same individuals. However, this was not carried out in this exposure assessment. It was therefore decided to use the mean body weights for the different age groups among children and adolescents in all calculations. If mean body weight is combined with individual data on consumption, the assessment may overestimate the degree of individual variation in dietary exposure, if their consumption is correlated with body weight.

7.3 Hazard identification and characterisation

7.3.1 Differences between rodents and humans

It is well known that cancer development in rodents and humans can be different, and that the interpretation of results from rodents to human health risk can be difficult. Normally, cancer development in rodents is used as an indicator test. This means that cancer development in any rodent organ after treatment with a chemical indicates that the chemical also can be a potential carcinogen in humans, if no specific conditions or strain specific knowledge indicate otherwise.

VKM regards the cholangiocarcinomas to be the most critical toxicological end point from furan exposure in rodents. Cancer in the biliary duct is known to be a malignant cancer type in humans. It has been suggested that there can be a tendency to overdiagnose the cholangiofibrosis and adenofibrosis as cholangiocarcinoma in rats. This has only been reported in a textbook without further references to detailed descriptions (Greaves, 2007). However, similar histopathology (intestinal differentiation) of the cholangiofibrosis and cholangiocarcinomas has also been reported by Elmore and Sirica 1993, indication that there might be some difficulties in separating cholangiocarcinomas from cholangiofibrotic lesions. The difference between rats and humans may contribute to an overestimation of the human risk from furan exposure in rats. Cholangiocarcinomas are normally not observed in untreated rats, and are not a frequent tumour in rats after chemical exposure. In addition, some cholangiocarcinomas in rats have been found to be malignant when transferred to recipient rats (Maronpot *et al.*, 1991). The cholangiocarcinomas have been characterised by an intestinal cell differentiation, which has been suggested as a risk factor in the development of cholangiocarcinomas in the biliary tract of humans (Elmore and Sirica 1993).

Rat and humans differs in the respect that rats do not have gallbladders, while humans have. It is not known what impact this difference will have on the toxicity of furan or whether it will affect the development of cholangiocarcinomas.

7.3.1 Differences between children and adults

Human hepatic CYP2E1 expression varies during human fetal liver development and in the early postnatal stage, but already in 90 days old infants the activity level was comparable to that in children and adults (Johnsrud *et al*., 2003). CYP2E1 demonstrates genetic polymorphism affecting the noncoding region of the gene, which may influence the transcription and expression of the gene (Chang and Kam, 1999). It has been shown for e.g. trichloroethylene that metabolism was altered with less than 2% under extreme values of CYP2E1 expression and activity (Lipscomb *et al*., 2003). It is unlikely that CYP2E1 variations could considerably alter furan metabolism, and they would probably not contribute considerably to the uncertainty in the risk assessment of furan.

It is expected that children can be more susceptible to genotoxic carcinogens than adults. Quantification analyses support the conclusion that there can be greater susceptibility for the development of tumours as a result of exposure to chemicals acting through a mutagenic mode of action, when the exposure occur in early life stage as compared with later life stages. Animal experiments starting exposure at week 6-7 will not take the increased susceptibility of children into account. This will contribute to en underestimation of the risk of furan exposure in children.

7.3.3 Limitations in data used for POD calculations

The most critical toxicological effect observed at the lowest furan dose (2 mg/kg bw/day) was the development of cholangiocarcinomas in male and female rats. The data on the development of cholangiocarcinomas in rats after 2 year of the NTP study (NTP, 1993) were not suitable for calculating a BMDL as a POD for furan toxicity, since there was close to 100% incidence at the lowest dose and therefore a dose-response for this toxic effect was lacking. As a sub-optimal solution VKM used the cholangiocarcinomas from the 9-month interim study (sex combined) to derive a POD, because these data showed a dose-response and were suitable for a BMDL calculation. Since the time point used in deriving the BMDL (9-months) is shorter than the full life span (2-year study) of the rat, a correction factor was used (see section 5.3.1). When taking into account both shorter exposure time and reduced observation time the correction factor is 7 and a POD of 0.02 mg/kg bw/day is derived. This would represent the most conservative POD based on the development of cholangiocarcinomas from the 9-month interim study.

7.4 Summary of uncertainties

Evaluations of the overall effect of identified uncertainties are presented in Tables 54 and 55, highlighting the main sources of uncertainty and indicating whether the respective source of uncertainty might have led to an over- or underestimation of the exposure and/or the resulting risk (EFSA, 2006b).

Table 54: Qualitative evaluation of influences of uncertainties on the assessment of furan exposure

Source of uncertainty	Direction
Dietary exposure assessment	
Different dietary assessment methods	+/-
Measurement uncertainty in the furan contents analysed	+/-

Source of uncertainty	Direction
Mean upper bound furan values used for foods from the EFSA furan database	+
Furan content in sweet breakfast cereals	+
Sped- and småbarnskost 2006/2007	
Portions of jarred baby food among 6-month-olds	+/-
Use of 95 percentile	+/-
FFQ time span is 14 days	+/-
Porridge powder with fruits	+
Ungkost 2000	
Study conducted in 2000-2001 - Possible changes in the food patterns can have occurred	+/-
Use of 95 percentile - The number of participants among 4-year-olds is only 391	+/-
Participation rate among 4-year-olds	+/-
Four registration days	+/-
Norkost 3, Adults	
Participation rate	+/-
Two registration days	+/-
Qualitative evaluation of overall effect of identified uncertainties:	+

+: uncertainty likely to cause over-estimation of exposure
-: uncertainty likely to cause under-estimation of exposure

VKM concluded that the furan exposure presented in this opinion can be considered realistic for each age group, despite of the limitations in assessing the food consumption and the uncertainties related to estimating the furan exposure outlined above. The exposure estimates are based on Norwegian food consumption data and primarily on furan concentrations for Norwegian food samples.

The similarities in histopathology between cholangiofibrosis and cholangiocarcinomas in rats with a possible over-diagnosis of the cholangiocarcinomas, may contribute to an overestimation of the human risk from furan exposure (see Table 55). As a sub-optimal solution, VKM used the cholangiocarcinomas from the 9-month interim study (sex combined), including a correction factor of 7, to derive a POD. This procedure will probably result in an overestimation of the risk related to furan exposure in humans. It is however noted that by comparing tumour incidence at 9 and 24 months this is increased by a factor of about 2.5 (Table 44).

Table 55: Qualitative evaluation of influences of uncertainties on evaluation of hazard in the risk assessment of furan

Source of uncertainty	Direction
Diagnosis of cholangiocarcinomas in rats	+
Use of the 9-months cholangiocarcinomas including a correction factor for calculation of POD	+
The lack of biliary bladder in rats	+/-
Children are probably more susceptible to genotoxic carcinogens	-
Qualitative evaluation of overall effect of identified uncertainties:	+

+: uncertainty likely to cause over-estimation of hazard
-: uncertainty likely to cause under-estimation of hazard

Data gaps

- The only available 2-year carcinogenicity study in rats (NTP, 1993) is not suitable for deriving a POD, since the incidence of cholangiocarcinomas was already 100% at the lowest dose. A new 2-year study in rats with a selection of doses that provides a dose-response in cholangiocarcinomas is needed in order to perform an improved risk assessment of furan to humans. VKM is aware of an on-going 2-year carcinogenicity study in rats with exposure to low doses of furan performed by the FDA. The present risk assessment on furan may need to be updated when data from this study become available.
- *In vivo* studies on genotoxicity of BDA, the main metabolite of furan, should be performed since genotoxicity has been found in several *in vitro* tests. Such *in vivo* studies may also contribute to elucidate the mechanism behind the *in vivo* genotoxicity of furan.
- A small number of samples and food types has been analysed for furan contents. In addition data on furan concentrations in Norwegian coffee do not exist. This contributes to the uncertainties in the exposure calculations for furan. Since coffee is a major source of furan exposure in adults, new data on the furan concentrations in Norwegian coffee would improve the risk assessment for adults.
- A comprehensive and continually updated national furan database is necessary for estimating the overall exposure of furan through diet. Furan concentrations in both processed and heated foods are warranted.
- Further research and more data are needed on the presence of furan in home-cooked foods.
- More data is needed to understand under-/over-reporting of food consumption in dietary surveys.
- Further research is needed to get more accurate portion size estimations in the dietary surveys.
- Further research is needed to evaluate the impact of variations in number of registration days in the dietary surveys.

Conclusions

The liver is the main target organ for furan toxicity both in mice and rats, but the rat is the most sensitive species. A dose-dependent increase in hepatocellular adenomas and carcinomas was observed in mice and rats, and an increase in the incidence of cholangiocarcinomas was observed in rat liver. Cholangiocarcinomas in male and female rats are the most sensitive toxicological end point observed in rodents. On the basis of the available data, VKM considers that rat cholangiocarcinomas may be relevant for human risk from furan.

- Available *in vivo* data with furan indicate that a reactive metabolite, most likely BDA, is formed and that this metabolite can react with DNA and induce mutations. To VKM's knowledge, no *in vivo* studies on genotoxicity of BDA have been performed, but BDA was found genotoxic in several *in vitro* tests. VKM therefore considers that genotoxic mechanisms in furan-induced carcinogenesis cannot be excluded. Since a threshold cannot be identified, the substance was assessed as a genotoxic carcinogen.
- VKM used the Margin of Exposure (MOE) approach in this risk assessment. The suitability of different studies on cholangiocarcinomas for dose-response modelling was considered. The 9-month interim evaluation of a 2-year study from NTP (1993) was chosen because it demonstrates a dose-response relationship. From this study, a point of departure of 0.02 mg/kg bw/day was chosen, based on a benchmark dose lower bound ($BMDL_{10}$) of 0.14 mg furan/kg bw/day and a correction factor of 7 to account for reduced dosing and observation time.
- For 6-, 12- and 24-month-old children, the main source of furan exposure is jarred baby food. For 4-, 9- and 13-year-old children, the major food source to the furan exposure is breakfast cereals. In adults, the major contribution to the furan exposure is coffee. The highest furan exposure was calculated for 12-month-old infants and ranged from 0.62-1.51 µg/kg bw/day. In adults the furan exposure ranged from 0.27-0.82 µg/kg bw/day.
- For mean exposure among infants, children and adolescents, the MOE-values ranged from 29 in 12-month-infants to 2000 in the 13-year-old adolescents. Among high consumers in these groups, the MOE-values ranged from 13 to 400. In adults, the corresponding MOE-values ranged from 59 to 74 for mean furan exposure and from 24 to 26 for high exposure.
- It should be noted that the risk assessment of furan contains notable uncertainties and limitations, such as:
    - the use of the 9-month interim study in rats including a correction factor of 7 to derive a point of departure, instead of the full life-time study (2-year study) of the rat, is likely to overestimates the hazard.
    - a possible over-diagnosis of the cholangiocarcinomas, due to the similarities in histopathology between cholangiofibrosis and cholangiocarcinomas in rats may overestimate the hazard.
    - limitations in assessing food consumption and uncertainties related to estimating the furan exposure.

VKM considers that the current exposure to furan in all age groups, particularly among infants and children, is of health concern.

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Appendices

Appendix I

DIETARY EXPOSURE TO FURAN FROM JARRED BABY FOOD IN 6-MONTH-OLD NON-BREASTFED INFANTS (SPEDKOST 2006)

Table A1 shows the consumption of different subcategories jarred baby food and exposure to furan 6 month-old non-breastfed infants. The mean and 95 percentile consumptions of jarred baby food are calculated based on the frequency of consumption reported in the dietary survey and the amount of baby food assumed to be consumed per meal (1/4 jar = ca 50 g) (Øverby *et al*., 2009).

The furan exposures have been calculated based on the mean furan contents in the different subcategories of jarred baby food analysed as purchased (see Table 1 and 6) and on the mean and 95 percentile consumptions shown in Table A1. A calculation of the furan exposure from the total of jarred baby food consumed has also been included, based on the individual consumption data reported for 6-month-old non-breastfed infants. All values in Table A1 are given for consumers only among the non-breastfed participants (n=397) in the dietary survey.

Table A1: Consumption of different subcategories jarred baby food and exposure to furan in 6-month-old non-breastfed infants (n=397). Values for consumers only within each subcategory are given.

		Frequency of consumption, times/day		Amount consumed, g	Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Baby food subcategory	**Number of consumers (% of 397)**	**Mean (SD)**	**95 percen-tile**	**1/4 jar of baby food**	**Mean (SD)**	**95 percen-tile**	**Mean**	**95 percen-tile**
Meat and vegetables	214 (54)	0.8 (0.3)	1.0	50	39 (17)	50	0.19	0.24
Vegetables	138 (35)	0.6 (0.4)	1.0	50	32 (18)	50	0.13	0.20
Fish and vegetables	48 (12)	0.6 (0.3)	1.0	50	28 (16)	50	0.11	-[c]
Fruits[b]	274 (69)	0.9 (0.6)	2.0	50	46 (30)	100	0.05	0.10
Total jarred baby food	331 (83)	1.6 (0.9)	3.3	50	80 (45)	165	**0.23**	**0.49**

[a] Calculated based on the mean body weight of 8.0 kg reported for 6-month-old infants (see Table 7).
[b] Include berries but not juice.
[c] Few consumers (0.38 µg/kg bw/day)

The results in Table A1 should be compared with the furan exposures shown for all participants in the dietary survey in Table 8.

DIETARY EXPOSURE TO FURAN FROM JARRED BABY FOOD IN 12-MONTH-OLD NON-BREASTFED INFANTS (SPEDKOST 2007)

Table A2 shows the consumption of different subcategories jarred baby food and exposure to furan in 12-month-old non-breastfed infants. The furan exposures have been calculated based on the mean furan contents in jarred baby food analysed as purchased (see Table 1 and 6) and on the mean and 95 percentile consumption of the different subcategories of jarred baby food reported in the dietary survey. A calculation of the furan exposure from the total of jarred baby food consumed has also been included, based on the individual consumption data reported for 12-month-old non-breastfed infants. All values in Table A2 are given for consumers only among the non-breastfed participants (n=881) in the dietary survey.

Table A2: Consumption of different subcategories jarred baby food and exposure to furan in 12-month-old non-breastfed infants (n=881). Values for consumers only within each subcategory are given.

		Consumption, g/day		Furan exposure, µg/kg bw/day[a]	
Baby food subcategory	**Number of consumers (% of 881)**	**Mean (SD)**	**95 percentile**	**Mean**	**95 percentile**
Meat and vegetables	659 (75)	115 (91)	277	0.45	1.08
Fish and vegetables	351 (40)	54 (30)	118	0.17	0.37
Cereal based	143 (16)	54 (33)	139	0.32	0.80
Vegetables	75 (9)	56 (40)	195	0.18	0.62
Fruits	608 (69)	81 (77)	218	0.07	0.18
Total jarred baby food	777 (88)	201 (158)	473	**0.58**	**1.33**

[a] Calculated based on the mean body weight of 9.9 kg reported for 12-month-old infants (see Table 7).
[b] Includes berries but not juice.

The results in Table A2 should be compared with the furan exposures shown for all participants in the dietary survey in Table 13.

Appendix II

Table A3: BMD and BMDL calculations on cholangiocarcinomas from the 9-month interim evaluation of NTP (1993), combining the sexes (all parameters shown from the calculation in Proast software)

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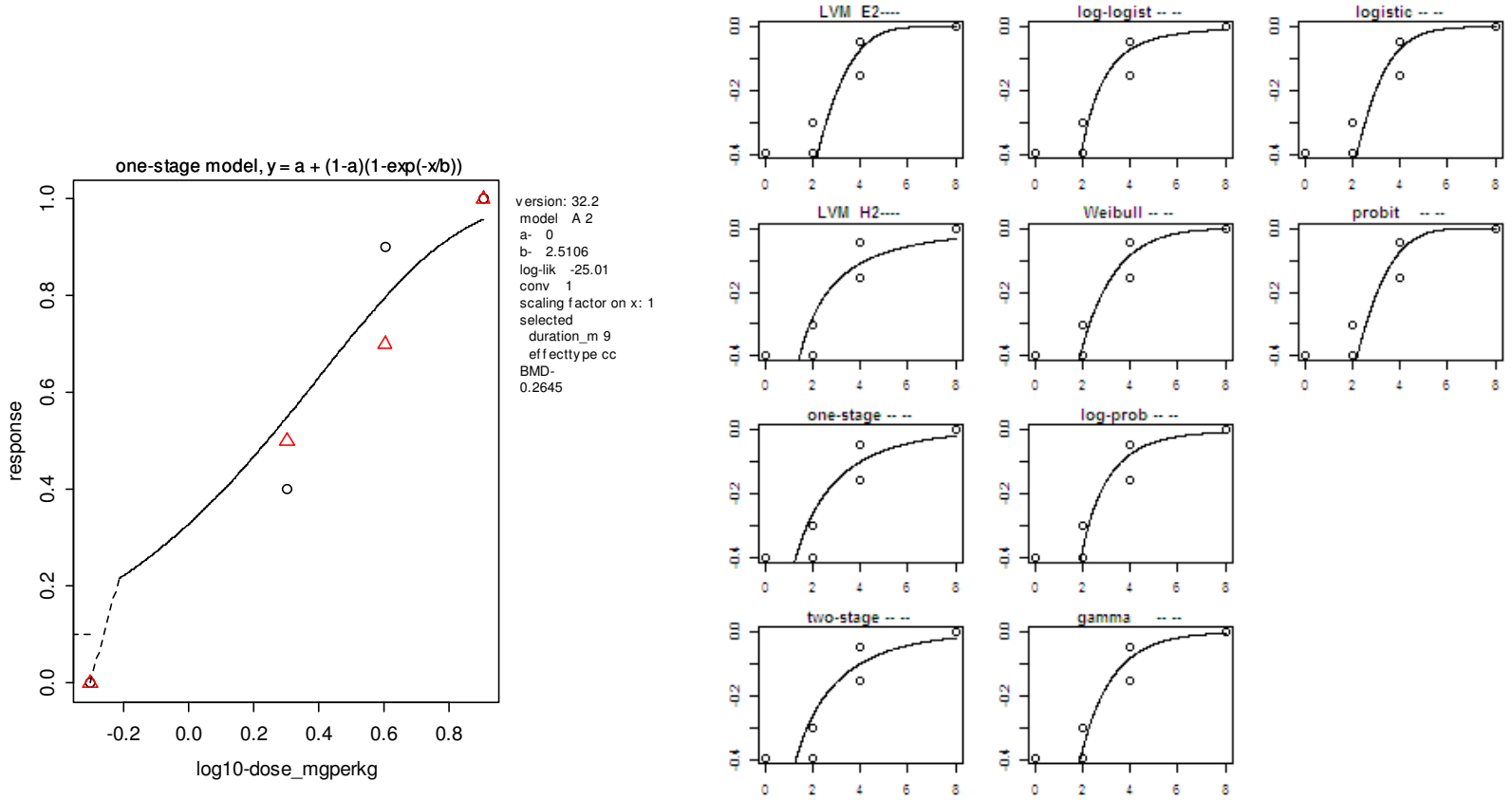
cholangiofibroses_carcinomas , response :

model	covar	npar	loglik	accept	BMD	BMDL	BMDU	level
null	NA	1	-54.83	--	NA	NA	NA	--
full	NA	8	-23.02	--	NA	NA	NA	--
one-stage	--	2	-25.01	yes	0.265	0.198	0.358	f
two-stage	--	3	-25.01	yes	0.265	0.198	0.358	f
log-logist	--	3	-24.43	yes	1.04	0.455	1.5	f
Weibull	--	3	-23.92	yes	0.713	0.216	1.28	f
log-prob	--	3	-24.2	yes	1.05	0.468	1.52	f
gamma	--	3	-24.01	yes	0.834	0.326	1.42	f
logistic	--	2	-25.4	yes	1.01	0.68	1.45	f
probit	--	2	-25.1	yes	0.704	NA	NA	f
LVM-E2		2	-25.1	yes	0.97	0.655	1.43	f
LVM-H2		2	-25.57	yes	0.484	0.349	0.663	f

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no

Figure A1: Plot of BMD calculations on cholangiocarcinomas from the 9-month interim evaluation of NTP (1993), combining the sexes.



Appendix III

CALCULATION OF T25

The T25 approach is defined as the chronic dose rate (usually expressed in units of mg per kg bodyweight per day) which will give tumours at a specific tissue site in 25% of the animals after correction for spontaneous incidence and within the standard life time of the species (Dybing *et al.*, 1997). In cases where the exposure/observation times are different from the standard lifespan of 24 months, the T25 value is corrected. The T25 method (Dybing et al., 1997, Sanner *et al.*, 2001) are used within EU in setting specific concentration limits for carcinogens in preparations (EC, 1999) and recently as a basis for calculation of Lifetime Cancer Risk and for quantitative hazard assessment of non-threshold carcinogens in several regulatory areas e.g. ECHA (2010), SCHER/SCCP/SCENIHR (2009) and SCCS (2010).

In the case of furan, the tumour frequency after 24 months was close to 100% (NTP, 1993). In such cases the T25 will be uncertain and the "true" T25 will probably be lower than the estimated T25 after 24 months. It is, however, possible to use the 9 and 15 months interim study in the calculation. The calculated T25 in the interim studies has to be divided by a factor "d" (Dybing *et al.*, 1997; EC, 1999; ECHA, 2010; Carcinogen Potency Database, 2011).

d = (24 months / exposure time) x (24 months / observation time)

This imply that the uncorrected T25 after 9 months is divided with ([24/9] x [24/9]) = 7.11 and the uncorrected T25 after 15 months is divided with ([24/15] x [24/15]) = 2.56. The results of the calculation of T25 are shown in Table A4. As expected, T25 after 24 months is much higher both for females and males than the T25 values calculated after 9 months and 15 months. The average exposure value after 9 and 15 months gives T25 = 0.14 mg/kg bw/day.

Table A4: T25 calculation on cholangiocarcinomas from the 9-, 15- and 24-month evaluation of NTP (1993). The exposure doses have been calculated as the daily exposure.

Observation time/exposure time (months)	Females				T25 Females Uncorrected mg/kg bw/d	T25 Females Corrected [a] mg/kg bw/d
	Control	1.4 mg/kg bw	2.9 mg/kg bw	5.7 mg/kg bw		
9	0/10 (0%)	4/10 (40%)	9/10 (90%)	10/10 (100%)	0.89	0.13
15	0/9 (0%)	9/10 (90%)	9/9 (100%)	7/7 (100%)	0.40	0.16
24	0/50 (0%)	49/50 (98%)	50/50 (100%)	48/50 (96%)	0.36	-
Observation time/exposure time (months)	**Males**				**T25 Males Uncorrected mg/kg bw/d**	**T25 Males Corrected [a] mg/kg bw/d**
	Control	**1.4 mg/kg bw**	**2.9 mg/kg bw**	**5.7 mg/kg bw**		
9	0/10 (0%)	5/10 (50%)	7/10 (70%)	10/10 (100%)	0.71	0.10
15	0/9 (0%)	7/9 (78%)	9/9 (100%)	6/6 (100%)	0.46	0.18
24	0/50 (0%)	43/50 (86%)	48/50 (96%)	49/50 (98%)	0.42	-

[a] T25 is corrected for observation/exposure time and is divided with ([24/9] x [24/9]) = 7.11 after 9 months, and with ([24/15] x [24/15]) = 2.56 after 15 month.