Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment of coumarin intake in the Norwegian population

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

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SUMMARY

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has at the request of the Norwegian Food Safety Authority (Mattilsynet) conducted a risk assessment of the coumarin intake in the Norwegian population. VKM was asked to assess if any part of the population has a total intake of coumarin that will exceed the tolerable daily intake (TDI). It should further be considered whether an intake of coumarin exceeding TDI 1-2 times a week for several years would represent a risk to the health of the consumer. The assessment has been performed by the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics (Panel 4).

Coumarin is a naturally flavouring substance in cinnamon and occurs in many plants. The substance can be found in different types of cinnamon to a varying degree. The two main types are Ceylon (*Cinnamomum zeylandicum*) and Cassia cinnamon (*Cinnamomum aromaticum*). Cassia cinnamon, which currently is most frequently used in food products on the Norwegian market, contains more coumarin than the lesser used Ceylon cinnamon.

Oral intake of coumarin is mostly related to consumption of cinnamon-containing foods or cinnamon as a spice. This includes both direct addition of cinnamon to foods as well as the use of cinnamon oils and other cinnamon extracts by the food industry. Other important sources of exposure could be food supplements based on cinnamon or the use of cosmetic products through dermal exposure, as synthetic coumarin is added as a fragrance ingredient to perfumes, skin gels, lotions and deodorants.

It is known from animal experiments that coumarin can cause liver toxicity. It is considered as a non-genotoxic carcinogen in mice and rats. In 2004, the European Food Safety Authority (EFSA) established a TDI of 0.1 mg coumarin/kg body weight (bw), based on a no observed adverse effect level (NOAEL) for liver toxicity in a 2-year dog study. This TDI was maintained when the substance was re-evaluated in 2008. EFSA further concluded that exposure to coumarin resulting in an intake 3 times higher than the TDI for 1-2 weeks was not of safety concern.

In order to answer the second question as stated in the terms of reference, the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics found it necessary to further examine the data on toxicity of coumarin, which were the basis for the TDI established by EFSA. The most significant hazards of coumarin appears to be liver toxicity, which is well documented, and demonstrated in mice, rats, dogs, baboons and humans, and kidney adenomas in male rats. In a review of human case reports, a small subgroup of the human population appears for unknown reasons to be more susceptible to medical treatment with coumarin. The lowest reported dose of coumarin associated with liver toxicity in humans is around 0.4 mg/kg bw/day. It should be noted that the liver toxicity of coumarin in humans usually is reversible. Since there were no dose-response data for humans, animal data were used in the hazard characterisation.

The VKM Panel decided to use the benchmark dose (BMD) approach to determine a point of departure for adverse effects of coumarin. The 2-year chronic toxicity/carcinogenicity study in rats by the US National Toxicology Program (NTP) was chosen for model simulation and BMD/BMDL (benchmark dose lower confidence limit) calculations. The best model fit of the dose-response data combined with the lowest $BMDL_{05}$ (dose where the response is likely to

be smaller than 5%) was seen for increased relative liver weight in female rats, which gave a $BMDL_{05}$ of 7 mg/kg bw/day (converted from 10 mg/kg bw, 5 times per week).

The VKM Panel used the $BMDL_{05}$ for relative increase in liver weight in female rats to establish a TDI of 0.07 mg/kg bw/day using an uncertainty factor of 100 to account for inter- and intraspecies variation.

The intake calculations for coumarin from food and drinks in this opinion are based on both data from the nationally representative food consumption surveys Norkost, Ungkost, Småbarnskost and Spedkost, as well as on assumed worst intake scenarios of different cinnamon-containing food products. The average coumarin levels found in cinnamon-containing food categories such as ginger bread, cinnamon buns and similar bakery products, cinnamon-containing cakes, thin pastry with cinnamon and cinnamon-based tea sold on the Norwegian market, were used to calculate the total coumarin intake in different age groups in the population. For the calculation of the coumarin intake from cinnamon powder sprinkled on oatmeal porridge and rice porridge, a coumarin level of 3000 mg/kg in cinnamon powder was used. The frequency of consumption and the amount of cinnamon powder (from ¼ - 1 teaspoon) sprinkled on the porridge were taken into account in the calculations.

To assess if any part of the Norwegian population has an intake of coumarin that will exceed the TDI, the different intake scenarios presented in the opinion have been compared with the TDI of 0.07 mg/kg bw/day established by VKM. The main conclusions from the VKM Panel were:

The total estimated intake of coumarin for mean and high consumers of cinnamon-containing foods are below the TDI for all age groups when consumption of cinnamon-based tea and porridge with cinnamon was excluded.

Children and adults who regularly consume oatmeal porridge sprinkled with cinnamon may exceed the TDI by several folds depending on the frequency of consumption and the amount of cinnamon used.

Small children (1- and 2-years old) who have a mean or high consumption of oatmeal porridge may exceed the TDI even if they use moderate amounts of cinnamon powder on the porridge. In a worst case scenario with high consumption of porridge and use of high amounts of cinnamon powder, the estimated coumarin intake could exceed the TDI by about 20-fold. This intake is similar to dose levels of coumarin used in medical treatment of adults and where cases of liver toxicity have been reported.

Drinking of cinnamon-based tea, which may have a high content of coumarin, can also result in a total intake of coumarin that exceeds the TDI both for children and adults.

Other relevant sources of coumarin are cosmetics and food supplements with cinnamon. The recommended dose of two cinnamon supplements sold on the Norwegian market can lead to an exceedance of TDI in adults. It is not anticipated that children will consume supplements with cinnamon. Cosmetic products (shower gels, body lotions, deodorants and oils) are important sources of coumarin exposure both for children and adults, but quantification of the coumarin exposure from cosmetics was not possible due to lack of data.

The VKM Panel concludes that based on the available data, the possibility of an adverse health effect by exceeding the TDI 3-fold for 1-2 times per week for several years cannot be assessed. Generally, a minor or an occasional exceedance of TDI is not considered to increase the risk of adverse health effects.

The coumarin intake could exceed the TDI by 7-20 fold in some instances. Liver toxicity may occur shortly after the start of coumarin exposure. Such large daily exceedances of TDI, even for a limited time period of 1-2 weeks, cause concern of adverse health effects.

NORSK SAMMENDRAG

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet gjennomført en risikovurdering av inntaket av kumarin i den norske befolkningen. VKM ble bedt om å vurdere om noen deler av befolkningen har et totalinntak av kumarin som vil representere en overskridelse av det tolerable daglige inntaket (TDI) for stoffet. Videre skulle det vurderes om en overskridelse av TDI 1-2 ganger i uken gjennom flere år ville kunne representere en helsefare for forbruker. Vurderingen er gjort av VKMs faggruppe for tilsetningsstoffer, aroma, matemballasje og kosmetikk (Faggruppe 4).

Kumarin er en naturlig aromatisk bestanddel av kanel og finnes også i mange planter. Stoffet finnes i varierende grad i forskjellige typer kanel. De to hovedtypene er Ceylonkanel (*Cinnamomum zeylandicum*) og Cassiakanel (*Cinnamomum aromaticum*). Cassiakanelen, som i dag er mest i bruk på det norske markedet, inneholder mer kumarin enn den mindre brukte Ceylonkanelen.

Oral eksponering for kumarin kommer hovedsaklig fra inntak av kanelholdig mat og drikke eller kanel som krydder. Dette omfatter både direkte tilsetning av kanel til en matvare og bruk av kaneloljer eller andre kanelekstrakter i næringsmiddelindustrien. Andre viktige kumarinkilder kan være kanelbaserte kosttilskudd (kaneltabletter), eller opptak gjennom huden som følge av bruk av kosmetiske produkter. Syntetisk kumarin tilsettes som en aromatisk ingrediens i parfymer, hudkremer, lotions og deodoranter.

Det er kjent fra dyreforsøk at kumarin kan forårsake levertoksisitet. Stoffet er betraktet som et ikke-gentoksisk karsinogen i mus og rotter. EFSA, EUs mattrygghetsorgan, fastsatte i 2004 en TDI-verdi på 0,1 mg kumarin/kg kroppsvekt, basert på en nulleffektsdose (NOAEL) for levertoksisitet fra en 2-årsstudie på hunder. Denne TDI-verdien ble opprettholdt når stoffet ble revurdert i 2008. EFSA konkluderte i sin siste vurdering også med at et kumarininntak 3 ganger over TDI 1-2 uker i strekk ikke medfører helsefare.

For å svare på det andre spørsmålet i oppdraget fra Mattilsynet, fant VKMs faggruppe for tilsetningsstoffer, aroma, matemballasje og kosmetikk det nødvendig å vurdere toksisitetsdataene for kumarin, som er basis for den nåværende TDI-verdien fastsatt av EFSA, nærmere. De viktigste endepunktene for kumarintoksisitet synes å være levertoksisitet, noe som er vel dokumentert og vist i mus, rotter, hunder, bavianer og mennesker, samt nyreadenomer hos hannrotter. En gjennomgang av rapporter på enkelttilfeller (kasusrapporter) viser at en liten gruppe mennesker av ukjente årsaker synes å være mer følsomme for kumarin brukt i medisinsk behandling. Den laveste dosen av kumarin som er forbundet med levertoksisitet i mennesker er omkring 0,4 mg/kg kroppsvekt/dag. Levertoksisiteten av kumarin hos mennesker antas vanligvis å være reversibel. Siden det ikke finnes noen dose-

respons data for mennesker, ble data fra dyreforsøk benyttet i risikovurderingens farekarakterisering.

VKMs faggruppe besluttet å bruke benchmark dose (BMD)-modellering for å fastsette et nivå for når negative effekter av kumarin inntreffer. En 2-års-toksisitets/karsinogenisitetsstudie i rotter, utført av National Toxicology Program (NTP) i USA, ble valgt for modellsimuleringer og BMD/BMDL (benchmark dose nedre konfidensnivå)-beregninger. De dose-respons-dataene som viste seg å være best tilpasset modellen, kombinert med å gi den laveste $BMDL_{05}$-verdien (den dosen som gir en lavere respons enn 5%), ble funnet for økt relativ levervekt i hunnrotter. Beregningen gav en $BMDL_{05}$-verdi på 7 mg/kg kroppsvekt/dag (omregnet fra en dose på 10 mg/kg kroppsvekt, gitt 5 ganger i uken).

VKMs faggruppe har benyttet den beregnede $BMDL_{05}$-verdien for relativ økning i levervekt hos hunnrotter til å fastsette en TDI-verdi på 0,07 mg/kg kroppsvekt/dag ved å bruke en usikkerhetsfaktor på 100 som tar høyde for variasjon mellom ulike arter og innad i samme art.

Inntaksberegningene for kumarin fra mat og drikke i denne risikovurderingen er basert både på data fra de nasjonale representative kostholdsundersøkelsene Norkost, Ungkost, Småbarnskost og Spedkost og på antatte verste-fall-inntaksscenarioer av ulike kanelholdige matvarer. Det gjennomsnittlige kumarinnivået som har blitt påvist i kanelholdige matvarekategorier som pepperkaker, kanelboller og lignende bakevarer, krydderkaker med kanel, smurte lefser med kanel og kanelholdig te solgt på det norske markedet, ble benyttet til å beregne det totale kumarininntaket hos ulike aldersgrupper av befolkningen. I beregningene av hvor mye kumarin man kan få i seg fra kanel brukt på havregrøt og risgrøt, ble det benyttet et kumarinnivå på 3000 mg/kg kanelpulver. Det ble tatt hensyn til hvor ofte man spiser grøt og hvor mye kanel som strøs på grøten (fra ¼ til 1 teskje) i beregningene.

For å kunne vurdere om noen deler av den norske befolkningen har et inntak av kumarin som overskrider TDI, ble de ulike inntaksscenarioene i risikovurderingen sammenlignet med TDI-verdien på 0,07 mg/kg kroppsvekt/dag fastsatt av VKM. Hovedkonklusjonene fra VKMs faggruppe var:

Det totale estimerte inntaket av kumarin blant forbrukere med et gjennomsnittlig og høyt inntak av kanelholdige matvarer er under TDI for alle aldersgrupper når konsumet av kanelholdig te og grøt med kanel ikke var tatt med i beregningene.

Barn og voksne som spiser havregrøt med kanel regelmessig kan overskride TDI betydelig, avhengig av hvor ofte de spiser grøt og mengden kanel som strøs på grøten.

Små barn (1- og 2-åringer) med et gjennomsnittlig eller høyt konsum av havregrøt kan overskride TDI selv om de bare bruker moderate mengder kanel på grøten. Ett verste-fall-scenario med et høyt konsum av grøt og bruk av store mengder kanel, resulterer i at det estimerte inntaket av kumarin kan overskride TDI med ca. 20 ganger. Dette inntaket tilsvarer de dosenivåer av kumarin som har blitt benyttet i medisinsk behandling av voksne personer og hvor tilfeller av levertoksisitet har blitt rapportert.

Enkelte typer kanelbasert te kan ha et høyt innhold av kumarin. Konsum av disse kan også resultere i et totalinntak av kumarin som overskrider TDI både hos barn og voksne.

Andre relevante kumarinkilder er kosmetiske produkter og kanelbaserte kosttilskudd (kaneltabletter). Inntak av anbefalt dose av to typer kaneltabletter som selges på det norske markedet kan føre til en overskridelse av TDI hos voksne. Det er antatt at barn ikke tar kaneltabletter. Kosmetiske produkter (dusjsåpe, kremer, body lotion, deodoranter og oljer) er en viktig kilde til eksponering for kumarin både hos barn og voksne, men på grunn av manglende data var det ikke mulig å kvantifisere kumarineksponeringen fra kosmetikk.

VKMs faggruppe konkluderer med at mulige skadelige helseeffekter ved en 3 gangers overskridelse av TDI 1-2 ganger i uken gjennom flere år ikke kan vurderes på bakgrunn av de tilgjengelige data. Små eller sporadiske overskridelser av TDI er generelt sett ikke antatt å øke risikoen for at det kan oppstå skadelig helseeffekter.

Inntaket av kumarin kan overskride TDI fra 7-20 ganger i enkelte tilfeller. Levertoksisitet kan inntreffe tidlig etter eksponering for kumarin. Slike store daglige overskridelser av TDI, selv i en begrenset tidsperiode på 1-2 uker, kan gi bekymring for skadelige helseeffekter.

KEY WORDS

Coumarin, cinnamon, liver toxicity, kidney adenomas, benchmark dose (BMD), benchmark dose lower confidence limit (BMDL), tolerable daily intake (TDI), cinnamon-containing foods, oatmeal porridge, rice porridge, cinnamon-based tea, food supplements, cosmetics.

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BACKGROUND

Coumarin is a naturally flavouring substance in cinnamon and occurs in many plants. The substance can be found in different types of cinnamon to a varying degree. The two main types are Ceylon (*Cinnamomum zeylandicum*) and Cassia cinnamon (*Cinnamomum aromaticum*). Cassia cinnamon, which currently is most frequently used in food products on the Norwegian market, contains more coumarin than the lesser used Ceylon cinnamon.

It is known from animal experiments that coumarin can cause liver toxicity. It is considered a carcinogen in mice and rats. In 2004, EFSA concluded that the carcinogenic effect was not caused by a genotoxic mechanism, and a tolerable daily intake (TDI) of 0.1 mg coumarin/kg body weight (bw), based on a no observed adverse effect level (NOAEL) for liver toxicity in a 2-year dog study was established (EFSA, 2004). Based on new available toxicity data, EFSA re-evaluated coumarin in 2008. It was then concluded to maintain the TDI of 0.1 mg coumarin/kg bw allocated in the 2004 opinion. EFSA further concluded that exposure to coumarin resulting in an intake 3 times higher than the TDI for 1-2 weeks is not of safety concern (EFSA, 2008).

The presence of coumarin in food products is not going to be banned as it is an important flavouring part of cinnamon. Currently, there exist a maximum level of coumarin, but this is so low (2 mg/kg food) that it in practice is difficult/impossible to comply with. According to regulation no. 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods, the European Commission has recently adopted new maximum levels for coumarin in certain compound food. The new restrictions shall apply from 20 January 2011. The maximum levels are:

Compound food in which the presence of the substance is restricted	***Maximum level***
Traditional and/or seasonal bakery ware containing a reference to cinnamon in the labelling	50 mg/kg
Breakfast cereals including muesli	20 mg/kg
Fine bakery ware, with the exception of traditional and/or seasonal bakery ware containing a reference to cinnamon in the labelling	15 mg/kg
Desserts	5 mg/kg

The Norwegian Food Safety Authority (Mattilsynet) has in 2008 and 2009 conducted two small surveys to investigate the level of coumarin in some selected food products on the Norwegian market. The results show that most of the food products complied with the new maximum levels that will apply from 2011. The new adopted restrictions were subject to extensive discussions in the EU Commission Working Group on Flavourings as it is assumed that the consumers do not eat a lot or have a long-term consumption of the food compounds with the highest maximum coumarin levels. Moreover, no maximum level of coumarin in pure cinnamon is established. The Norwegian Food Safety Authority has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess if any part of the Norwegian population has an intake of coumarin that will exceed the TDI. The presence of coumarin in food supplements and cosmetics should be taken into consideration in the exposure assessment.

TERMS OF REFERENCE

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety to assess the following:

- Does any part of the Norwegian population have a total intake of coumarin that will exceed the TDI?
    - For instance: children who regularly eat oatmeal porridge with cinnamon for breakfast, or who have large intake of other products containing cinnamon.
    - For instance: adults who have a large intake of products containing cinnamon.
- According to EFSA's last risk assessment, exposure to coumarin resulting in an intake 3 times higher than the TDI for 1-2 weeks is not of safety concern. However, will an intake of coumarin exceeding the TDI 1-2 times a week for several years represent a risk to the health of the consumer?

ASSESSMENT

In order to answer the second question as stated in the terms of reference, the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics found it necessary to further examine the data on toxicity of coumarin, which were the basis for the TDI established by EFSA.

HAZARD IDENTIFICATION

TOXICOKINETICS

General description of the metabolic pathways of coumarin

There is extensive information on the disposition and biotransformation of coumarin in different animal species and humans, which have been reviewed by several authors (Lake *et al*., 1999, Felter *et al*., 2006) and in opinions by the Scientific Committee of Food (SCF) and later by the European Food Safety Authority (EFSA) (EFSA, 2004; EFSA, 2008). The main metabolic pathways of coumarin are briefly described here based on these previous reviews.

Coumarin is rapidly absorbed from the gasterointestinal tract and distributed throughout the body following oral exposure. The compound appears to be extensively metabolised in all species with little unchanged coumarin being excreted. The routes of elimination of coumarin metabolites show considerable variations between species. It seems that in rats a considerable amount of the coumarin metabolites is excreted in the faeces (up to approximately 40%), while in humans coumarin metabolites are almost exclusively excreted in the urine, with non-detectable amounts in the faeces (Lake *et al*., 1999).

Two major biotransformation pathways exist for coumarin in addition to several minor pathways, as shown in Figure 1.

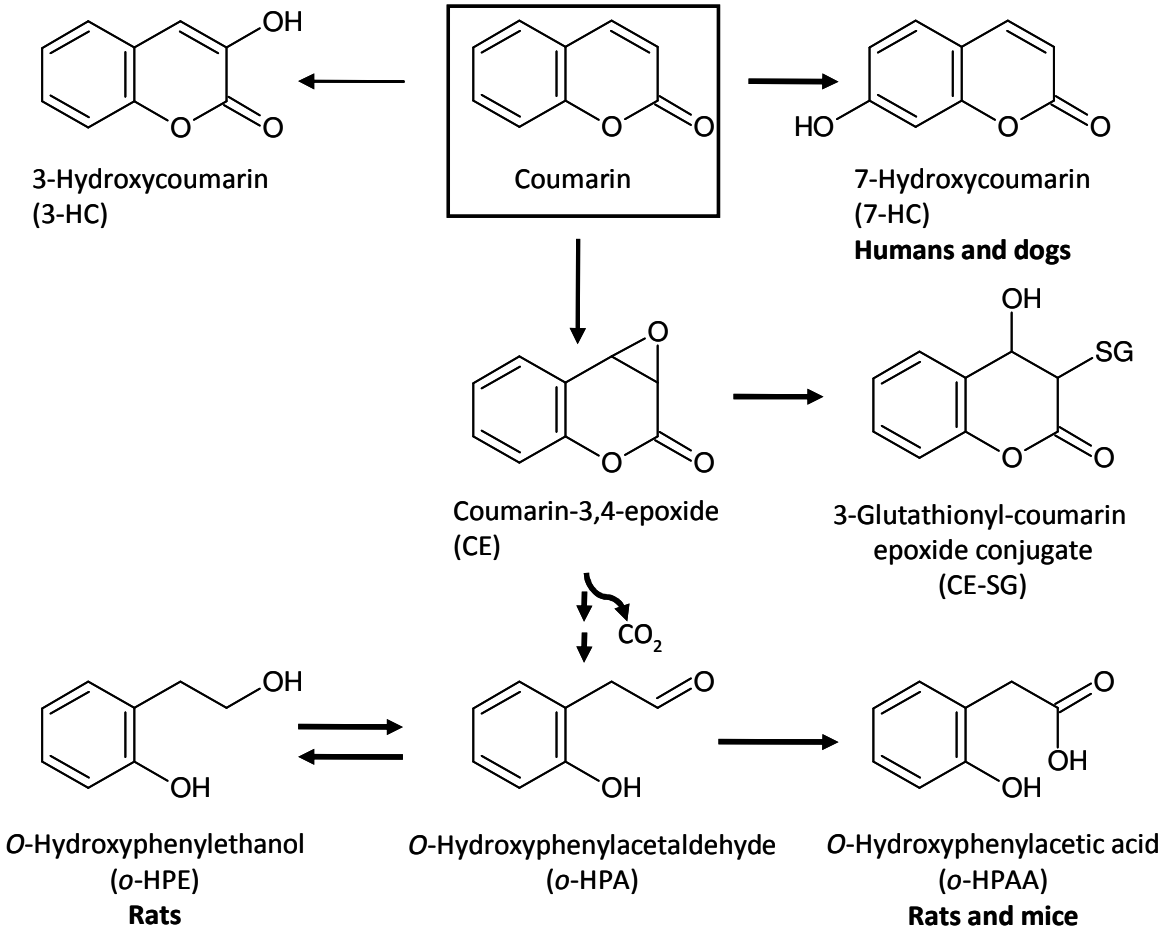

Figure 1. *The major metabolic pathways of coumarin biotransformation after oral exposure (adapted from EFSA 2004).*

The two most important pathways of coumarin metabolism after oral exposure are 7-hydroxylation to 7-hydroxycoumarin (7-HC) and epoxidation to the coumarin 3,4-epoxide intermediate (CE). Under aqueous conditions, coumarin 3,4-epoxide degrades rapidly with ring opening and loss of carbon dioxide to form *o*-hydroxyphenylacetaldehyde (*o*-HPA). *O*-HPA can be further reduced to *o*-hydroxyphenylethanol (*o*-HPE) or oxidised to *o*-hydroxyphenylacetic acid (*o*-HPAA). The formation of 3-hydroxycoumarin (3-HC) take probably place by direct hydroxylation, and not via the 3,4-epoxide, and it can be further metabolised to *o*-hydroxyphenyllactic acid (*o*-HPLA) (not shown). Hydroxylation of coumarin in the 4-, 5-, 6- and 8- position are very minor routes of metabolism. In general, the hydroxycoumarins are mainly excreted in the urine as glucuronic acid or sulphate conjugates.

Rats

In rats, various metabolites, including *o*-HPAA, are excreted in the faeces, and 3-HC, 7-HC, *o*-HPA and *o*-HPAA are found in the urine. 12-39% of the oral dose of coumarin is reported excreted in the faeces in rats. The 7-HC metabolite accounts for minor amounts

(approximately 0.7%), while *o*-HPAA is the dominating metabolite (Lake *et al*., 1999). Metabolites excreted in the faeces can be reabsorbed and contribute to a slow clearance of coumarin in rats. The intermediate epoxide CE converts rapidly to *o*-HPA and is further metabolised to *o*-HPAA and *o*-HPE. In addition, the rat has the ability to re-oxidize *o*-HPE to *o*-HPA, also contributing to a slow hepatic clearance of this toxic aldehyde. The half-life of coumarin in rats is 20 hours. *In vitro* studies with rat liver microsomes show that 40% of the total CE formed is converted to *o*-HPE (Vassallo *et al*., 2004). Studies demonstrate that 48% of a known amount of CE is detoxified in rats by liver cytosolic glutathione S-transferase to 3-glutathionyl-coumarin epoxide conjugate (CE-SG).

Mice

Up to 25% of the dose can be metabolised to 7-HC and excreted in the urine after intraperitoneal injection of coumarin in some strains, although other strains excrete considerably less 7-HC (2-3%) (Lake *et al*., 1999). In the B6C3F1 mouse strain approximately 7% of the oral coumarin dose of 200 mg/kg bw are metabolised to 7-HC (Felter *et al*., 2006). The major coumarin metabolite in mice is *o*-HPAA, which is formed from the conversion of the CE metabolite. In mouse, the half-life of coumarin is 4 hours. *In vitro* experiments with mouse liver microsomes show that 64% of the CE is converted to CE-SG in mice (Vassallo *et* al., 2004). In the same study, only very minor amounts of *o*-HPA is metabolised to *o*-HPE by mouse liver, and *o*-HPE is detected only at very high coumarin doses (1 mM).

Dogs

One *in vivo* study in dogs show that 3% of the administered oral dose of 200 mg/kg bw is excreted in the urine as 7-HC. Other metabolites were not studied (Gangolli *et al*., 1974).

Humans

Oral exposure

Coumarin is rapidly absorbed from the gastrointestinal tract and excreted in the urine in humans, with an elimination of 82% of the administered dose after 24 hours (Ritschel *et al*. 1977, Shilling *et al*., 1969; Egan *et al*., 1990). In the majority of human studies, coumarin is extensively metabolised to 7-HC and excreted in the urine. 7-HC and its glucuronide and sulphate conjugates are non-toxic and represent 40-92% of the urinary metabolites following a 200 mg oral dose in humans, while 4% (range 1-6%) are excreted as *o*-HPAA after 24 hours (Shilling *et al*., 1969; Egan *et al*., 1990). In a recent study, four subjects were given an oral dose of 2 mg coumarin and the urine was collected for 8 hours. Three of the subjects excreted mainly 7-HC, while the last subject excreted only 0.03% of the dose as 7-HC, but instead 50.3% as the metabolite *o*-HPAA (Hadidi *et al*., 1997). Inter-individual differences in human coumarin metabolism are reported, with some subjects excreting only 10% and 20% of the dose as 7-HC within the experimental period (Cholerton *et al*., 1992; Rautio *et al*., 1992; van Iersel *et al*., 1994).

Ritschel *et al*. (1977 and 1979) reported on the pharmacokinetics of per oral and i.v. administration of coumarin in man. The biological half-life of coumarin is comparable for both routes of administration, with 0.8 hours and 1.02 hours for oral and i.v. administration, respectively. The author reports that only 2-6% of coumarin reaches the systemic circulation intact after oral or i.v. administration, indicating an extensive first pass effect and efficient conjugation and elimination of coumarin. From the curve showing blood concentrations of

coumarin versus time, the peak plasma concentration of coumarin after oral exposure occurred after less than 15 minutes (Ritschel *et al*., 1977; Ritschel *et al*., 1979; Lake, 1999).

Dermal exposure

Coumarin was shown to be quickly absorbed, distributed and excreted in urine and faeces in humans (*n*=3 males) after dermal application of 0.2% [4-^{14}C]-coumarin in 70% alcohol to a skin surface of 100 cm^2 for 6 hours. Peak plasma radioactivity was 0.5 - 1 hour, and the half-life of coumarin was approximately 1.7 hours. Urinary and fecal excretion was 58.6% and 1.1%, respectively, after 120 hours. Coumarin was primarily metabolized to and excreted in urine as 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulphate. Small amounts of unconjugated 7-hydroxycoumarin and *o*-HPAA were also excreted (Ford *et al*., 2001).

Yourick and Bronaugh (1997) reported *in vitro* absorption on human abdominal skin of coumarin in ethanol solution to be 64% after 24 hours. They further reported absorption from an oil/water emulsion vehicle to be as high as 97% with human abdominal skin. Furthermore, higher *in vitro* absorption has been reported from human scalp skin than from abdominal skin (Ritschel *et al.*, 1989).

In vitro studies

In a study with liver microsomes from 12 humans, on average approximately 5% of the metabolites accounted for the coumarin-3,4-epoxide pathway, although there were individual variations (van Iersel *et al*., 1994). This is in accordance with the *in vivo* studies in humans. It is shown in several studies that P450 CYP2A6* is involved in 7-hydroxylation of coumarin, and that some polymorphic variants of CYP2A6* have reduced capacity for 7-hydroxylation of coumarin and therefore produce more *o*-HPAA via the coumarin-3,4-epoxide pathway (SCF, 1999).

In a recent study on physiologically-based biokinetic (PBBK) modelling, Rietjens *et al*. used results obtained with liver microsomes from rats and two different human donors having high and low 7-hydroxylation capacity (Rietjens *et al*., 2007; 2008 cited in EFSA, 2008). The Vmax and Km values for the different metabolic pathways were induced in the models. By setting the 7-hydroxycoumarin pathway to zero, the PBBK is supposed to mimic the *in vivo* situation of a person that is homozygous for the polymorphic allele CYP2A6*. A 70-fold increase in the *o*-HPA metabolite level was estimated in comparison with humans with the wild-type variant. Although this is only model estimations, the results from the PBBK model support the results from *in vivo* studies, i.e. a sub-population of humans will metabolise considerable amounts of coumarin to *o*-HPA.

Comparison of metabolism between species and route of exposure

Due to enterohepatic circulation in rats the half-life of coumarin and/or its metabolites are longer in rats than in humans. Humans excrete coumarin mainly in the urine. It may be anticipated that mice also have some degree of enterohepatic circulation due to the molecular weight of the coumarin metabolites, although we have found no documentation to support this. In addition, rats metabolise considerable amounts of *o*-HPA to *o*-HPE, which can be re-converted to *o*-HPA. In mice and humans, negligible amounts of *o*-HPE are formed and almost all of the *o*-HPA are oxidised directly to *o*-HPAA. Also contributing to the slow clearance of o-HPA in rat liver is the 20 to 50 times lower oxidation rate to o-HPAA compared to mice and humans (Felter *et al*., 2006, EFSA, 2004). Altogether, slower clearance of *o*-HPA is probably the major reason for the higher sensitivity of rats to liver toxicity from coumarin.

In the study with liver microsomes, it was found that mice produce CE-SG at twice the rate compared to rat liver microsomes (Vassallo *et al*., 2004), showing that mice produce CE at higher rate than rats. This can be a possible explanation for the higher sensitivity of mice to adenoma development in the liver compared to rats.

Since humans have a 50 times higher clearance of *o*-HPA than rats, and since there are no indication of enterohepatic circulation of coumarin in humans, humans are probably less sensitive to coumarin toxicity than both mice and rats. This could even be the case for humans with homozygous polymorphism of CYP2A6*.

It is likely that the balance between bioactivation (epoxide formation and rearrangement to *o*-HPA) and detoxification (glutathione conjugation of the epoxide and oxidation/reduction of *o*-HPA) dictates the *in vivo* susceptibility of a species to coumarin mediated liver toxicity and/or adenoma development in the liver.

The difference in peak plasma concentration occurrence of coumarin after oral and dermal exposure indicates that the absorption of coumarin after dermal exposure is slower than after oral exposure (Ritschel *et al*., 1977, Ford *et al*., 2001). Therefore, the acute toxicity of coumarin might differ, depending on the route of exposure. Abraham *et al*. (2010) have suggested that hepatic peak concentrations of coumarin are expected to be much lower after dermal compared with oral exposure to the same dose, due to slower absorption and the fact that the first pass phenomenon does not apply to dermal exposure. Accordingly, coumarin would be much less hepatotoxic after dermal compared to oral exposure, if hepatotoxicity is a threshold effect depending on the peak concentrations, but not if hepatotoxicity is related to the area under the curve (Abraham *et al.*, 2010).

Summary of toxicokinetics

Coumarin is rapidly absorbed in humans both after oral and dermal exposure. The substance is rapidly eliminated after administration, which is consistent with an absence of enterohepatic circulation of the metabolites in humans compared to rats. The major metabolite in humans is 7-HC and its glucuronide- and sulphate conjugates. The half-life of coumarin is comparable independent of route of administration (oral 0.8 hours, i.v. 1.02 hours and dermal 1.7 hours). Only 2-6% of coumarin reaches the systemic circulation intact after oral administration, indicating an extensive first pass effect and efficient conjugation and elimination of coumarin.

GENERAL TOXICITY

Acute toxicity

Mice

In various mouse strains acute oral LD50 of coumarin has been reported to be in the range of 196-780 mg/kg bw, and single oral doses (200 mg/kg bw) produced liver necrosis and increased plasma transaminase activities (Lake, 1999; Cottrell *et al*., 1996).

Born *et al*. (1998) administered coumarin by oral gavage at doses of 10, 20, 50, 100, 150 and 200 mg/kg bw to B6C3F1 mice to determine if coumarin is acutely toxic to Clara cells in the lung. The two highest doses of 150 and 200 mg/kg bw resulted in swelling and necrosis of Clara cells in the terminal bronchioles of male and female B6C3F1 mice.

Rats
The acute LD50 for oral exposure is 290-680 mg/kg bw in various rat strains. In rats, single oral or i.p. exposure of 125-500 mg/kg bw causes acute hepatotoxicity as assessed by histological and biochemical changes (Lake, 1999).

Born *et al*. (1998) administered coumarin by oral gavage at doses of 10, 20, 50, 100, 150 and 200 mg/kg bw to Fisher 344 (F344) rats to determine if coumarin is acutely toxic to Clara cells in the lung. Clara cell toxicity was less pronounced in the rats than in mice.

Other species
In guinea pig, the acute LD50 for oral exposure is 202 mg/kg bw (IARC 2000).

Subacute toxicity

Mice
In a 16-day study (NTP, 1993), groups of five male and five female B6C3F1 mice received coumarin in corn oil by gavage at doses of 0, 40, 75, 150, 300 or 600 mg/kg bw, 5 days a week, for a total of 12 doses in a 16-day period. All mice receiving 600 mg/kg bw, two male mice receiving 300 mg/kg bw, and one male mouse receiving 75 mg/kg bw died. The mean body weight gains and final mean body weights of surviving dosed male and female mice were similar to those of the controls. Clinical findings of inactivity, excessive lacrimation, piloerection, bradypnea, ptosis, or ataxia were observed in some mice from the 300 and 600 mg/kg bw groups within the first several hours after dosing. There were no clinical signs of organ-specific toxicity, and there was no evidence of impaired blood coagulation from measurements of capillary clotting time or prothrombin and activated partial thromboplastin time. Capillary clotting time and platelet counts of dosed mice were similar to those of controls.

Rats
Male rats were given 15, 45, 135 or 405 mg/kg bw/day of coumarin for seven days by oral intubation. There was no increase in relative liver weight at the two lower doses. However, there was a dose-related increase at the two highest doses. Histological changes occurred at the highest dose only and consisted of fatty change and vacuolar degeneration in the centrilobular hepatocytes. Dose-related depression in cytochrome P-450 and amidopyrine demethylase also occurred at the two highest doses (Grasso *et al*., 1974).

Male Wistar rats were given either 0 or 145 mg/kg bw of coumarin dissolved in arachis oil daily for seven days by gavage. The animals were sacrificed 24 hours after the last dose and liver samples were prepared for light and electron microscopy. There was a 33% reduction in the number of hepatocytes and about a 40% increase in the mean volume of the hepatocytes in the dosed rats. The mean smooth endoplasmic reticulum membrane area per g of liver significantly decreased in the coumarin-treated animals (De La Iglesia *et al*., 1975).

In a 16-day study (NTP, 1993) groups of five male and five female F344/N rats received coumarin in corn oil by gavage at doses of 0, 25, 50, 100, 200 or 400 mg /kg bw, 5 days per week for a total of 12 doses in a 16-day period. All female rats and four male rats receiving 400 mg/kg bw died. The mean body weight gains and final mean body weights of surviving dosed male and female rats were similar to those of the controls.

Dogs
One male and one female dog were given 100 mg/kg bw of coumarin by capsules six days per week for up to 16 days (Hagan *et al*., 1967). The male was killed in extremis after nine days and the female was found dead on day 16. Marked emaciation and slight dehydration and jaundice were noted. Macroscopically the livers were yellow coloured and had a nutmeg appearance. Microscopically there was marked disorganization of the lobular pattern, moderate increase in the size of the liver cells, vacuolation, a large amount of diffusely distributed fat, focal necrosis, fibrosis and very slight to moderate bile duct proliferation.

Subchronic toxicity

Mice
Male CD-1 mice were fed a diet containing 0.1-1% coumarin (approximately 50-500 mg/kg bw/day) for 13 weeks. No toxic effect of coumarin treatment was observed (Lake and Grasso, 1996).

In a 13-week study (NTP, 1993), groups of ten male and ten female B6C3F1 mice received coumarin in corn oil by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw, 5 days per week. Two male mice receiving 300 mg/kg bw died. The mean body weight gain and final mean body weight of surviving male mice that received 300 mg/kg bw were significantly lower than those of the controls. No clinical signs of toxicity were observed. Male and female mice receiving coumarin exhibited dose-related decreases in mean erythrocyte volume and mean erythrocyte hemoglobin. The absolute and relative liver weights of males and females that received 150 and 300 mg/kg bw were significantly greater than those of the controls. Centrilobular hepatocellular hypertrophy was observed in male and female mice receiving 300 mg/kg bw.

Rats
Male Sprague-Dawley CD rats were fed a diet containing 0.1-1% coumarin (approximately 50-500 mg/kg bw/day) for 13 weeks. In groups of 5-8 rats, coumarin produced dose-related hepatotoxic effects, which included vacuolar degeneration, apoptosis, and bile duct proliferation, and sustained stimulation of hepatocyte replicative DNA synthesis. These effects were particularly marked at dose levels of 0.3 and 0.5% (150 and 250 mg/kg bw/day) (Lake and Grosso, 1996).

In a 13-week study (NTP, 1993), groups of ten male and ten female F344/N rats received coumarin in corn oil by gavage at doses of 0, 19, 38, 75, 150, or 300 mg/kg bw, 5 days per week. Three male and three female rats receiving 300 mg/kg bw died. The mean body weight gains and final mean body weights of male rats that received 150 and 300 mg/kg bw were significantly lower than those of the controls. There were no clinical signs related to specific organ toxicity. Male and female rats receiving coumarin exhibited dose-related decreases in mean erythrocyte volume ($P<0.05$, in males at 38 mg/kg bw) and mean erythrocyte hemoglobin ($P<0.05$, in males at 19 mg/kg bw) and dose-related increases in erythrocyte counts ($P<0.05$, in females at 19 mg/kg bw). A dose-related decrease in mean level of serum cholinesterase was observed in males and females ($P<0.05$, 10-18% decrease at 19 mg/kg bw); this effect was most pronounced in females with a 60% decrease at 300 mg/kg bw (see Figure 2). Serum levels of total bilirubin and one or more cytoplasmic enzymes including alanine aminotransferase, aspartate aminotransferase, ornithine carbamoyltransferase, and/or sorbitol dehydrogenase in males and females receiving 300 mg/kg bw were higher than those of controls. The absolute and relative liver weights of male and female rats that received 150

and 300 mg/kg bw were significantly greater than those of the controls. Centrilobular hepatocellular degeneration and necrosis, chronic active inflammation, and bile duct hyperplasia were observed in the liver of rats receiving 150 or 300 mg/kg bw.

Hamsters
Male Syrian hamsters were fed a diet containing 0.1-1% coumarin (approximately 50-500 mg/kg bw/day) for 13 weeks. No toxic effect of coumarin treatment was observed (Lake and Grasso, 1996).

Chronic toxicity

Mice
In a 2-year study, Carlton *et al*. (1996) administered CD-1 mice a diet containing 0, 300, 1000, or 3000 ppm coumarin for 2 years (equivalent to intakes of 0, 26.2, 85.8, or 280 mg/kg bw/day in males and 0, 28, 91.3 or 271 mg/kg bw/day in females). No dose-related abnormalities in clinical signs, clinical pathology, haematology, or gross or microscopic pathology were noted. A marginal increase in liver weight was observed in female mice, but the weight increases were not statistically significant and no dose-related histopathology was reported. These results indicated a NOAEL of 3000 ppm (280 mg/kg bw/day for males, 271 mg/kg bw/day for females).

In a 2-year chronic toxicity/carcinogenicty study by the NTP (NTP, 1993), B6C3F1 mice were administered 0, 50, 100 or 200 mg/kg bw of coumarin by gavage 5 days/week. The principal toxic lesions associated with the administration of coumarin to mice occurred in the liver. The incidences of centrilobular hypertrophy in 100 and 200 mg/kg bw males and 200 mg/kg bw females were significantly greater than those of controls. The incidences of syncytial alteration in all male dose groups and in 200 mg/kg bw females were also significantly greater than controls. The incidences of eosinophilic foci, a putative preneoplastic lesion, and of hepatocellular adenoma were significantly greater in the 50 and 100 mg/kg bw females. The reason for a lack of liver response in 200 mg/kg bw female mice is not known, but the NTP indicated that it may be due in part to the decrease in body weight. The NTP bioassay showed hepatotoxicity in male mice at all dose levels. A lowest observed adverse effect level (LOAEL) of 50 mg/kg bw (37 mg/kg bw/day) for increased incidence of eosinophilic foci in liver could be identified: in females, the net increase was 36% ($P<0.01$); in males, the increase was 20% ($P<0.05$).

Rats
In a 2-year toxicity/carcinogenicity study, Carlton *et al*. (1996) administered Sprague-Dawley rats a diet containing 0, 333, 1000, 2000, 3000 or 5000 ppm coumarin (equivalent to intakes of 0, 13, 42, 87, 130 or 234 mg/kg bw/day in males and 0, 16, 50, 107, 156 or 283 mg/kg bw/day in females). Rats receiving 333, 1000 or 2000 ppm coumarin were exposed to these dose levels *in utero* and during the lactation period, then chronically following weaning. Rats in the 3000 and 5000 ppm dose groups received only post-weaning chronic exposure. All male rats were terminated after 104 weeks of post-weaning exposure, whereas female rats were terminated after 110 weeks. Survival was significantly decreased in male rats at 333 ppm, but significantly increased in the 3000 and 5000 ppm dose groups compared to the control. Dramatic dose-related decreases in body weight gain were recorded for rats receiving 2000, 3000, or 5000 ppm, clearly indicating that the MTD (maximum tolerated dose, as indicated by a body weight decrement of greater than 10-15%) was exceeded. Food consumption also was decreased at the three highest dose levels, although body weight

decrement was disproportionately large compared to changes in food consumption. The 1000 ppm dose caused a slight, but statistically significant reduction in body weight gain. Significant clinical findings included a treatment-related anemia, principally characterised by low haemoglobin levels among males and females from week 6 onward. This effect was noted primarily among rats in the three highest dose groups. Treatment-related decreases in glucose and protein were found at week 4 and 13 among 5000 ppm dose group animals. Treatment-related increases in blood potassium levels, alkaline phosphatase, and glutamic-pyruvic transaminase, were observed throughout the study (in which groups were not given in the report). Cholesterol levels of all groups of treated rats, except for males in the highest dose group, were increased throughout the study. Plasma cholinesterase levels, but not red blood cell cholinesterase levels, were decreased among females treated with coumarin, but not males. Unfortunately, the clinical chemistry data were not presented quantitatively in tables or figures, and the levels of statistical significance were not given. Without giving quantitative data it was also reported weight changes for a number of organs (brain, pituitary, heart, kidney, adrenal) within the groups exposed to 1000-5000 ppm. Quantitative data, but no statistical analyses, were presented for liver weights. Using the given means, standard deviations and group size, the VKM Panel calculated a statistical significant increase ($P<0.001$, *t*-test) in liver weight at 333 ppm for females (15% increase) and 3000 ppm for males (21% increase). Altogether, it was not possible to identify a NOAEL. This study indicated that LOAEL for effects on liver weight was 333 ppm for female rats (16 mg/kg bw/day).

In a 2-year chronic toxicity/carcinogenicty study by the NTP (NTP, 1993), F344 rats were administered 0, 25, 50 or 100 mg/kg bw of coumarin in corn oil by gavage 5 days/week. After 15 months, 10 animals from each group were evaluated. In this evaluation, the values for one or more hematologic parameters including mean erythrocyte volume, mean erythrocyte hemoglobin in 50 and 100 mg/kg bw rats, and hematocrit or hemoglobin in 100 mg/kg bw rats were significantly lower than those of controls. Activated partial thromboplastin times were also significantly lower in 50 and 100 mg/kg bw males, while platelet counts were significantly higher. Female and male rats showed a dose-dependent increase in absolute and relative liver weight. In females, there was a 10% statistically significant increase of relative liver weight with 25 mg/kg bw. Activities of alanine aminotransferase, sorbitol dehydrogenase, or γ-glutamyltransferase in 50 and 100 mg/kg bw male and 100 mg/kg bw female rats were significantly higher than those of the controls at the 15-month interim evaluation. The rest of the animals were treated with coumarin in two years. None of the male rats receiving 100 mg/kg bw and only two males receiving 50 mg/kg bw survived until the end of the study (vehicle control, 28/50; 25 mg/kg bw, 9/50; 50 mg/kg bw, 2/51; 100 mg/kg bw, 0/50). Survival of dosed female rats was similar to that of the controls (vehicle control, 29/50; 25 mg/kg bw, 38/50; 50 mg/kg bw, 36/50; 100 mg/kg bw, 30/50). The reduced survival in dosed male rats was primarily attributed to coumarin-related exacerbation of spontaneously occurring renal disease. Among male rats, there was a statistically significant dose-dependent decrease in survival (trend analysis, $P<0.001$). Even at 25 mg/kg bw, there was a 5% decrease in survival ($P<0.001$). The principal lesions associated with the administration of coumarin to rats for up to two years occurred in the liver, kidney and forestomach. While the hepatic lesions were seen in all groups of males, they occurred only in the 50 and 100 mg/kg bw females. The lesions consisted of a spectrum of changes including hepatocellular necrosis, fibrosis, cytologic alteration and increased severity of bile duct hyperplasia. There was a chemical-related increase in the average severity of nephropathy in all groups of dosed male and female rats. There were corresponding increased incidences of parathyroid gland hyperplasia in all groups of dosed males, probably as a result of

compromised renal function. The incidences of forestomach ulcers in all groups of dosed male rats and in 100 mg/kg bw female rats were significantly greater than those of the controls (males: vehicle control, 7/48; 25 mg/kg bw, 24/50; 50 mg/kg bw, 35/51; 100 mg/kg bw, 34/50; females: vehicle control, 1/48; 25 mg/kg bw, 1/49; 50 mg/kg bw, 6/50; 100 mg/kg bw, 9/48). For male and female rats, a LOAEL of 18 mg/kg bw/day (converted from 25 mg/kg bw, 5 times per week) could be identified. For males, this LOAEL represents 24% net increase (incidence treated group – incidence control/1 – incidence treated group) in incidence of liver necrosis ($P<0.01$), 45% increase in the incidence of severity grade of nephropathy ($P<0.01$), 39% net increase in the incidence of forestomach ulcer ($P<0.01$), and 5 % decrease in days of survival ($P<0.001$). For females, this LOAEL represents 15% increase in the incidence of nephropathy ($P<0.05$), 33% in the incidence of severity grade of nephropathy ($P<0.05$), and 10% increase of liver weight ($P<0.05$).

Dogs

Hagan *et al*. (1967) administered oral capsules of coumarin 6 days/week to dogs at doses of 10 mg/kg bw/day (2/sex) for 297–350 days; 25 mg/kg bw/day (2/sex) for 133–330 days; 50 mg/kg bw/day (2 males and 1 female) for 35–277 days; and 100 mg/kg bw/day (1/sex) for 9–16 days. Doses of 25 mg/kg bw/day and higher were reported to produce liver damage, with an indicative NOAEL of 10 mg/kg bw/day (8.6 mg/kg bw/day). In this study, the number of individuals was few and the pathological effects were not consistent, e.g. at 25 mg/kg bw/day there was moderate emaciation in one female but weight gain in the other three dogs. Macroscopically, the liver was "slightly pale in two dogs, dark red in a third, and yellow, markedly 'nutmeg' in the fourth". However, no definite effects were seen at 10 mg/kg bw/day, and various effects were described in all the other groups. In this report no statistical analyses were performed and the results were not discussed.

Baboons

In a 2-year study (Evans *et al*., 1979), groups of four male baboons were fed a diet providing 0, 2.5, 7.5, 22.5 or 67.5 mg/kg bw/day of coumarin. Like humans, this primate metabolizes coumarin extensively by the 7-hydroxylation pathway. Relative liver weight was only significantly increased in baboons given 67.5 mg/kg bw/day of coumarin. While light microscopic examination of liver sections revealed no abnormalities, ultrastructural examination revealed a dilatation of the endoplasmic reticulum in three of four animals given 67.5 mg/kg bw/day of coumarin. A NOAEL of 22.5 mg/kg bw/day was identified in this study.

Human studies

EFSA (2008) and BfR (Abraham *et al.*, 2010; Bergmann, 1999) have reviewed the literature on adverse health effects of coumarin in humans. Coumarin has since the seventies been used as a medical drug for the treatment of oedema, varicose veins, cancer, infections, chronic fatigue syndrome etc., sometimes in combination with troxerutin, which is claimed to protect against oxidative liver damage. As a consequence of numerous reported cases of serious hepatotoxicity, coumarin was withdrawn from the marked in some countries.

Hepatotoxicity associated with treatment with coumarin has been observed in clinical studies and there are a number of case reports. The doses of coumarin used were 25-200 mg coumarin daily, but even higher doses have been given. In a study by Cox *et al*. (1989), 2173 patients were given 25-2000 mg coumarin per day, with a majority receiving 100 mg/day for one month and then 50 mg/day for two years. Reversible hepatotoxicity attributed to coumarin was found in 0.37% of the pasients. In another study on 140 women who received 400 mg

coumarin daily, 6 % developed hepatotoxicity, which was reversible (Loprinzi *et al*., 1999). Review of the cases has attributed many of the cases reported as idiosyncratic or unpredictable adverse drug reaction affecting a subgroup of the population. The proportion of the population belonging to this group has been estimated to be less than 10% (Bergmann, 1999).

Among the 82 cases of reported liver damage associated with coumarin-administration (Bergmann, 1999), it was possible to undertake oral dose classification for 51 cases. The VKM Panel calculated that the median dose was 90 mg/day and the 5 percentile was 30 mg/day. The lowest dose causing hepatotoxicity was 25 mg/day (equivalent to 0.4 mg/kg bw/day), comprising 4% of the cases. Notably, it is not possible to describe a dose-response relationship because the frequency of cases at each dose is not known.

Considering all available human data, Abraham *et al.* (2010) concluded that underlying mechanisms of coumarin-related hepatotoxicity in the susceptible human subgroup has not yet been elucidated, and that evidence for a genetic polymorphism of CYP2A6* with deficient 7-hydroxylation of coumarin as the cause of high sensitivity is missing. As long as this polymorphism is not demonstrated to be linked to the coumarin-susceptible subgroup, other possible mechanisms have to be considered as well, for instance immune mechanisms.

However, they conclude that evidence of coumarin hepatotoxicity in a subgroup of the human population is striking and has to be considered in risk assessment of coumarin, whatever the underlying mechanism may be. The case reports evaluated by Bergmann (1999) allowed an estimation of the time period critical for the onset of hepatitis in sensitive individuals. The shortest period documented were 5 days with 90 mg/day and 16-18 days with 30 mg/day. This demonstrates fast response in sensitive individuals and possible health risk when exceeding TDI over several weeks.

GENOTOXICITY

In vitro

Bacteria tests

The mutagenicity of coumarin has been examined in *Salmonella typhimurium* and found to be negative in TA98, TA1535, TA1537 and TA1538 with or without metabolic activation. Coumarin was not mutagenic in TA100 without metabolic activation and with non-activated rat liver S9-mix. However, when Aroclor 1254-induced rat or hamster liver S9-mix was used, coumarin induced a concentration-related mutagenic effect up to 2000 µg/plate. At higher concentrations (2500 and 3000 µg/plate) toxic effect was observed (Haworth *et al*., 1983). In the Haworth *et al*. study, there was a 1.9-fold increase in revertants with Aroclor-induced rat liver S9-mix, whereas there was a 3.3-fold increase with Aroclor-induced hamster liver S9-mix. In the NTP report from 1993, coumarin was also found to be mutagenic in *Salmonella typhimurium* strain TA100 in the precence of metabolic activation, but not in the absence of metabolic activation. In accordance with the Haworth *et al*. study, the highest mutagenic potency was observed with Aroclor-induced hamster liver S9-mix. A 2.5-fold increase in revertants was observed with S9-mix from hamster and 1.8-fold increase with S9-mix from Aroclor 1254-treated rats (NTP, 1993). Another positive effect was reported in an abstract by Norman and Wood (1981), with a 2-fold increase in revertants with S9-mix from Aroclor 1254-treated rats, but not when S9-mix from untreated rats or mice was used. In this study,

chemical analyses of metabolites were also reported, and it was claimed that mice liver metabolised coumarin at a 6-fold faster rate than rat liver S9. The studies would support the involvement of the CE pathway in the induction of gene mutations. A positive response was also reported by Stoltz and Scott (1980) in TA100 with metabolic activation, but the magnitude of the response was not reported. Coumarin was found negative in TA100 in two spot tests conducted with metabolic activation (Florin *et al*. 1980; Rhodia, 1978). The studies with positive effects were performed as plate incorporation or preincubation studies, which are also recommended in the current OECD guidelines for mutagenicity testing in bacteria.

Cell cultures

In Chinese hamster ovary (CHO) cells, coumarin at doses giving maximum 50% toxicity (50-500 µg/ml without S9-mix and 160-1600 µg/ml with S9-mix) induced sister chromatid exchanges (SCE) in the absence, but not in the presence of activation with S9-mix from Aroclor 1254-treated rats. The effect did not appear to be concentration-dependent (Galloway *et al.* 1987). In another study with CHO cells, coumarin (50-500 µg/ml without S9-mix and 160-1600 µg/ml with S9-mix) induced a concentration-dependent increase in chromosomal aberrations (from 5% in controls to 37% in the highest dose) in the presence, but not in the absence of metabolic activation by S9-mix from Aroclor 1254-treated rats. A coumarin induction of SCE was observed without S9 activation, but not with S9 activation. However, the increase in SCE was not dose-related (NTP, 1993). Sasaki *et al*. (1987) found no evidence of SCE or chromosome aberration (CA) in cultured CHO cells treated with coumarin at doses of 0, 3.3, 10, 33, 100 and 333 µM.

Organ culture

In cultured human liver slices from four individuals, coumarin concentrations up to 5mM had no effect on unscheduled DNA synthesis (Beamand *et al*. 1998).

In vivo

Mice

In B6C3F1 mice given 75, 150 or 300 mg/kg bw coumarin by gavage 5 days/week for 13 weeks, no increased frequency of micronuclei in peripheral blood erythrocytes was observed. It should be noted that the performance of the micronucleus test by NTP is not performed according to OECD guidelines and are a part of a 13-week study. However, the dosing is continued until sampling and it is regarded as acceptable as long as toxicity has been demonstrated. No cytotoxicity in the bone marrow was reported (NTP, 1993). No increased micronuclei were observed in bone marrow cells from ICR mice administered 65 or 130 mg/kg bw coumarin by gavage for 7 days (Morris and Ward, 1992). These findings were consistent with the results of Api (2001), who demonstrated that orally administered coumarin (50, 100, or 200 mg/kg bw in 5 mice/sex) did not cause any increase in the incidence of micronucleated polychromatic erythrocytes (MPE) in the bone marrow of Swiss mice, whereas the positive control mitomycin C produced a significant increase. The mice were killed 24 hours or 48 hours after the treatment. Neither coumarin nor mitomycin C was cytotoxic to the bone marrow cells. However, sluggishness in two female mice in each group receiving 100 and 200 mg/kg bw of coumarin was observed, indicating systemic toxicity of coumarin at the two highest dose levels.

Rats
Oral administration of single doses of coumarin (32, 107 or 320 mg/kg bw) to male Sprague-Dawley rats did not induce unscheduled DNA synthesis (UDS) test in hepatocytes (Edwards *et al*., 2000). The maximum tolerated dose was 320 mg/kg bw.

The ability of coumarin to covalently bind to DNA in the target organs for tumour induction following long-term oral exposure was studied in the liver and kidney of male Sprague-Dawley and F344 rats. The Sprague-Dawley rats were dosed with 60, 120, or 240 mg/kg bw C14-radiolabeled coumarin (250 µCi/kg bw). The C14-radiolabel was located on the benzene ring. The F344 rats were dosed with 25, 50, or 100 mg/kg bw with the same radioactivity. Although a large amount of the C14-radiolabeled coumarin was present in the liver and kidney homogenates, there was no radioactivity in the DNA fractions, indicating no covalent binding to DNA (Swenberg, 2003; EFSA, 2004).

Drosophila melanogaster
Adults or larvae exposed to coumarin did not show sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (NTP, 1993).

Summary of genotoxicity
Positive effect of coumarin at high doses was observed in bacterial mutation assays. Negative results were reported in an UDS test in rats, and no adduct formation were found in rats after coumarin treatment. No induction of micronuclei was observed in three micronucleus tests *in vivo* in mice (Edwards *et al.,* 2000; NTP, 1993; Api, 2001). It had not been demonstrated in the micronucleus tests that the test compound had reached the bone marrow, although certain systemic effects were observed. In addition, the positive results on point mutation in a bacterial assay cannot be overruled by a negative result in the *in vivo* micronucleus test since this test measure another end-point than the bacterial assay. In should also be noted that the UDS test is performed in rats, while the liver adenomas are found in mice. It is therefore not possible to completely rule out a genotoxic mechanism of coumarin in mice, although the present results from genotoxicity tests in rats and mice suggest that coumarin are not genotoxic in rodents.

CARCINOGENICITY

Mice
In a 2-year chronic toxicity/carcinogenicity study by the NTP (NTP, 1993), groups of 50–51 male and 50–51 female B6C3F1 mice, six to seven weeks, were administered coumarin (purity > 97%) in corn oil by gavage at doses of 0, 50, 100 and 200 mg/kg bw 5 days per week for 103 weeks. Survival of all dosed groups was similar to that of the controls. Body weight gain was reduced in high-dose females.

There was significantly increased incidence of alveolar/bronchiolar adenomas at the 200 mg/kg bw dose in both males and females and a significant increased incidence of hepatocellular adenomas in females at the low and medium doses, but not at the highest dose. There was a significant increase in the incidence of squamous cell papillomas of the forestomach in the low-dose males and females (NTP, 1993) (Table 1).

***Table 1**. Incidences of tumors in mice in the 2-year gavage study of coumarin (NTP, 1993).*

Tumor site	Males				Females			
	Control	50 mg/kg	100 mg/kg	200 mg/kg	Control	50 mg/kg	100 mg/kg	200 mg/kg
Alvolar/bronchiolar								
Adenoma[a]	14/50 (28%)	8/50 (16%)	14/50 (28%)	**24/51 (47%)**	2/51 (4%)	5/49 (10%)	7/49 (14%)	**20/51 (39%)**
Carcinoma[b]	1/50 (2%)	1/50 (2%)	2/50 (4%)	1/51 (2%)	0/51 (0%)	0/49 (0%)	0/49 (0%)	**7/51 (14%)**
Adenoma or Carcinoma[c]	14/50 (28%)	9/50 (18%)	15/50 (30%)	**25/51 (49%)**	2/51 (4%)	5/49 (10%)	7/49 (14%)	**27/51 (53%)**
Hepatocellular								
Adenoma[d]	26/50 (52%)	29/50 (58%)	29/50 (58%)	27/51 (53%)	8/50 (16%)	**26/49 (53%)**	**29/51 (57%)**	12/50 (24%)
Carcinoma[e]	11/50 (22%)	11/50 (22%)	5/50 (10%)	3/51 (6%)	0/50 (0%)	3/49 (6%)	3/51 (6%)	1/50 (2%)
Adenoma or carcinoma[f]	35/50 (70%)	34/50 (68%)	31/50 (62%)	29/51 (57%)	8/50 (16%)	**27/49 (55%)**	**31/51 (61%)**	13/50 (26%)
Forestomach								
Squamous cell papilloma[g]	2/50 (4%)	**8/50 (16%)**	2/50 (4%)	0/50 (0%)	1/52 (2%)	**5(50 (10%)**	2/51 (4%)	2/51 (4%9
Carcinoma[h]	0/50 (0%)	1/50 (2%)	2/50 (4%)	0/50 (0%)	0/52 (0%)	1/50 (2%)	1/51 (2%)	0/51 (0%)
Papilloma or carcinoma[i]	2/50 (4%)	**9/50 (18%)**	4/50 (8%)	0/50 (0%)	1/52 (2%)	**6/50 (12%)**	3/51 (6%)	2/51 (4%)

Bold number: Significantly different ($P \leq 0.05$) from the control group by the logistic regression test.

Historical incidence for 2-year corn oil gavage studies with vehicle control groups (mean ± standard deviation):

[a]Male: 141/900 (15.7% ± 5.7%); range 4%-28% Female: 40/899 (4.4% ± 2.4%); range 0%-10%
[b]Male: 34/900 (3.8% ± 3.6%); range 0%-12% Female: 19/899 (2.1%± 2.0%); range 0%-6%
[c]Male: 166/900 (18.4%± 5.9%); range 6%-28% Female: 58/899 (6.5% ± 3.7%); range 0%-14%
[d]Male 249/901(27.6% ± 15.0%); range 4%-58% Female: 94/898 (10.5% ± 7.2%); range 2%-26%
[e]Male: 155/901 (17.2% ± 5.8%); range 8%-32% Female: 41/898 (4.6% ± 3.6%); range 0%-14%
[f]Male: 370/901 (41.1% ±15.5%); range 14%-72% Female: 129/898 (14.4% ± 8.1%); range 2%-34%
[g]Male: 27/902 (3.0% ±3.4%); range 0%-14% Female: 27/901 (3.0% ± 2.9%); range 0%-10%
[h]Male: 4/902 (0.4% ± 0.9%); range 0%-2% Female: 3/901 (0.3% ± 1.0%); range 0%-4%
[i]Male: 31/902 (3.4% ± 3.6%); range 0%-14% Female: 30/901 (3.3% ± 3.3%); range 0%-10%

In a 2-year toxicity and carcinogenicity study by Carlton *et al*. (1996), groups of 52 male and 52 female Swiss CD-1 mice, four weeks of age, were administered coumarin (purity > 98%) in the diet at concentrations of 0, 300, 1000 or 3000 mg/kg of diet (ppm) for either 101 weeks (males) or 109 weeks (females). The achieved intakes of coumarin for the three doses were 26, 86 and 280 mg/kg bw/day for males, and 28, 91 and 271 mg/kg bw/day for females, respectively. Survival did not differ between groups, although exact rates were not recorded. Body weight gain was significantly decreased by 18% in the high-dose males and by 10% in the mid-dose males, relative to the controls. The authors reported that there was no significant increase in the incidence of pulmonary adenocarcinomas in males (11/52 (21%) control, 17/52 (33%) low-dose, 10/52 (19%) mid-dose and 20/52 (38%) high-dose) and that the incidences were within the historical control range. Although there was an increased incidence of hepatocellular tumours (adenomas and carcinomas combined) in low-dose females (0/50 (0%) controls, 8/52 (15%) low-dose, 4/52 (8%) mid-dose and 3/52 (6%) high-dose females), the authors discounted these increases as being within the historical control range for that laboratory (Carlton *et al*., 1996).

Rats

In a 2-year chronic toxicity/carcinogenicity study by the NTP (NTP, 1993), groups of 50 male and 50 female Fischer 344/N rats, six to seven weeks of age, were administered coumarin (purity > 97%) in corn oil by oral gavage at doses of 0, 25, 50 and 100 mg/kg bw 5 days per week for 103 weeks. Survival of low-, mid- and high-dose males (9/50, 2/50 and 0/50 respectively) was significantly lower (P<0.001) than that of controls (28/50), as was body weight gain. In males, increased mortality was attributed to a chemical-related increased severity of renal disease in dosed animals. There was an increased severity of bile duct hyperplasia and renal tubule hyperplasia in both sexes. Although, the incidence of renal adenomas after conventional single-section evaluation was low, only up to 4%, this is a rare tumor and the occurrence in historical control groups has never been more than 2%. Consequently, it was decided to perform additional step sections of the kidneys. Approximately 6 to 8 additional sections were performed. Additional rats, primarily dosed males, were identified with renal tubule hyperplasia or adenoma (Table 2). An accompanying stop-exposure study was carried out in which groups of 20 male rats received 100 mg/kg bw/day of coumarin by gavage in corn oil for 9 or 15 months followed by corn oil gavage only until the end of the study at 103 weeks. Survival was 9/20 and 2/20 in the two groups, respectively. Whereas hepatic lesions including bile duct hyperplasia were reversible and the incidence of renal tubule adenomas, based on single sections, was not significantly increased, there was a significant increase in the 9-month 100 mg/kg bw dose group after step-sectioning of the kidney (4/20; 20%) but not in the 15-month group (2/20; 10%) (NTP, 1993).

Table 2. *Incidences of kidney tumors in rats in the 2-year gavage study of coumarin (NTP, 1993).*

Procedure	Males				Females			
	Control	25 mg/kg	50 mg/kg	100 mg/kg	Control	25 mg/kg	50 mg/kg	100 mg/kg
Single sections								
Adenoma[a]	1/49 (2%)	2/50 (4%)	2/51 (4%)	1/50 (2%)	0/49 (0%)	0/50 (0%)	0/50 (0%)	2/49 (4%)
Step sections								
Adenoma	0/49 (0%)	4/50 (8%)	**5/51 (10%)**	4/50 (8%)	0/49 (0%)	0/50 (0%)	1/50 (2%)	1/49 (2%)
Carcinoma	0/49 (0%)	1/50 (2%)	0/51 (0%)	0/50 (0%)	-	-	-	-
Adenoma or carcinoma	0/49 (0%)	**5/50 (10%)**	**5/51 (10%)**	4/50 (8%)	-	**-**	**-**	-
Single and step sections combined								
Adenoma	1/49 (2%)	**6/50 (12%)**	**7/51 (14%)**	5/50 (10%)	1/49 (2%)	0(50 (0%)	1/50 (2%)	2/49 (4%)
Carcinoma	0/49 (0%)	1/50 (2%)	0/51 (0%)	0/50 (0%)	-	-	-	-
Adenoma or carcinoma	1/49 (2%)	**6/50 (12%)**	**7/51 (14%)**	5/50 (10%)	-	-	-	-

Bold number: Significantly different ($P \leq 0.05$) from the control group by the logistic regression test.

Historical incidence for 2-year corn oil gavage studies with vehicle control groups (mean ± standard deviation):
[a]Male: 8/1019 (0.8% ± 1.0%); range 0%-2% Female: 2/1018 (0.2% ± 0.6%); range 0%-2%

In a 2-year toxicity and carcinogenicity study by Carlton *et al*. (1996), groups of 50 male and 50 female Sprague-Dawley rats were administered coumarin (purity > 98%) in the diet at concentrations of 0, 333, 1000, 2000, 3000 and 5000 mg/kg of diet (ppm) for 104 weeks (males) or 110 weeks (females). Groups receiving 3000 and 5000 ppm were 21–28 days of age at the beginning of the study, while the other treated groups were exposed to coumarin

diet *in utero* and throughout the postnatal and chronic periods. The achieved intakes of coumarin for the five doses were 13, 42, 87, 130 and 234 mg/kg bw/day for males, and 16, 50, 107, 156 and 283 mg/kg bw/day for females, respectively. Survival was less than 50% in all groups (including controls) except the groups receiving the two highest doses, in which it was between 50 and 70%. Dose-related decreases in body weight gain in excess of 10–15% occurred in the 2000, 3000 and 5000 ppm dose groups. Significantly increased incidences of cholangiocarcinomas, some of which were reported as metastasizing, were found in the highest-dose males and females (males: 0/65 (0%), 0/65 (0%), 0/65 (0%), 0/65 (0%), 1/65 (2%) and 37/65 (57%); females: 0/65 (0%), 0/65 (0%), 0/65 (0%), 0/65 (0%), 0/65 (0%) and 23/65 (35%), in the control, 333, 1000, 2000, 3000 and 5000 ppm dose groups respectively). Hepatocellular tumours were also found in high-dose males: 2/65 (3%), 2/65 (3%), 1/65 (2%), 1/65 (2%), 6/65 (9%) and 29/65 (45%) and females: 0/65 (0%), 0/65 (0%), 0/65 (0%), 0/65 (0%), 1/65 (2%) and 12/65 (18%) (Carlton *et al*., 1996).

Discussion and summary of carcinogenicity studies

Mice

Carlton *et al*. (1996) reported that while no hepatocellular tumors were found in the control female mice, 15% of the low-dose (28 mg/kg bw/day) and 8% of the mid-dose (91 mg/kg bw/day) had tumors. The authors concluded that the incidences were within laboratory historic control values. The working group of International Agency for Research on Cancer (IARC) noted that no information was provided on statistical evaluation in this paper (IARC, 2000). However, the IARC Working Group was aware of an unpublished company report in which statistical analyses had been applied to mortality-adjusted tumor rates. The Fisher's exact test for differences between treatment groups and Mantel's test for dose-related trends showed no treatment-related effect for any tumor type. The mice study by Carlton *et al*. (1996) will therefore not be further discussed.

NTP concluded that under the conditions of their 2-year study there was some evidence of carcinogenic activity of coumarin in male B6C3F1 mice based on the increased incidence of alveolar/bronchiolar adenomas. There was clear evidence of carcinogenic activity of coumarin in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and hepatocellular adenomas. The marginally increased incidences of squamous cell papillomas of the forestomach in male and female mice receiving 50 mg/kg bw/day may have been related to coumarin administration.

Rats

In rats, Carlton *et al*. (1996) found an increased incidence of cholangiocarcinomas and hepatocellular tumors at the highest doses given (234-283 mg/kg bw/day). It should be noted that a single oral exposure of rats to 125-500 mg/kg bw caused acute hepatotoxicity. Furthermore, the IARC Working Group (IARC, 2000) noted the unusually sharp increase in tumour incidence in the liver at only the highest of five doses and the lack of adequate histopathological description of both tumour types. Given the issue related to misdiagnosis of bile duct tumours in an earlier study, the IARC Working Group was concerned that no descriptive information or illustrations were provided to confirm the diagnosis of cholangiocarcinoma, nor a discussion of the pathology. In addition, tumors were only observed at doses toxic to the liver. Because of this and the lack of histopatological characterisation and also lack of *in vivo* liver genotoxicity in rats, the VKM Panel finds the rat study by Carlton *et al*. (1996) less relevant for risk assessment and it will therefore not be taken into further consideration.

Regarding the finding of renal tubule adenomas in the 2-year gavage studies in rats, NTP (1993) concluded that there was some evidence of carcinogenic activity of coumarin in male F344/N rats. There was equivocal evidence of carcinogenic activity of coumarin in female F344/N rats based on a marginally increased incidence of renal tubule adenomas.

HAZARD CHARACTERISATION

LOAEL AND NOAEL VALUES FROM ANIMAL TOXICITY STUDIES

LOAEL and NOAEL values from a series of long-term studies are presented in Table 3. Liver toxicity/pathology is the most common effect seen in the various species tested. Rats appeared to be the species most susceptible to coumarin, with a LOAEL of 16 mg/kg bw/day in female Sprague-Dawley rats (Carlton *et al*., 1996), and 18 mg/kg bw/day in male and female F344 rats (NTP, 1993) for effects on liver.

In order to compare doses from continuous dietary exposure (Carlton *et al*., 1996) with doses from 5 times/week by gavage exposure (NTP, 1993), the VKM panel has converted the gavage exposure to a continuous mg/kg bw/day measure. The LOAEL values are derived from the lowest dose showing a statistical significant effect as compared with controls. In Sprague-Dawley rats, the LOAEL of 16 mg/kg bw/day represents a 15% increase in liver weight observed among females in a 2-year study (Carlton *et al*., 1996). The LOAEL of 18 mg/kg bw/day in F344 rats derived from the NTP 1993 study is based on pathology data from the 2-year study, as well as liver weight recordings from the 15-month interim evaluation. This LOAEL represents: in females, 33% increased severity grade of nephropathy, 15% net increased incidence of nephropathy and 10% increase in liver weight; in males, 24% net increased incidence of liver necrosis, 45% increased severity of nephropathy, 39% net increased incidence of forstomach ulcer, as well as a 10% net increase in the incidence of kidney adenomas in male rats.

The effects in mice differed significantly between the strains tested: no effects were seen in CD-1 mice (Carlton *et al*., 1996); while a LOAEL of 37 mg/kg bw/day for liver pathology was identified in B6C3F1 mice in the 2-year NTP-study (NTP, 1993). This LOAEL represents: in females, 36% increased net incidence of eosinophilic foci in the liver and 46% increased net incidence of liver tumours; in males, 20% net increased incidence of eosinophilic foci in the liver.

NOAEL values of 8.6 mg/kg bw/day and 22.5 mg/kg bw/day were identified in dogs (Hagan *et al*., 1967) and baboons (Evans *et al*., 1979) respectively. However, these studies were old and with only four animals per group.

Table 3. *LOAEL and NOAEL values for chronic toxicity and carcinogenicity of coumarin in different species.*

LOAEL* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Species	Effect	Route frequency	Gender N/group	Reference
37		B6C3F1 Mice	Net increased (36%) incidence of eosinophilic foci in the liver	gavage 5 times/week	F 50	NTP, 1993
37		B6C3F1 Mice	Net increased (20%) incidence of eosinophilic foci in the liver	gavage 5 times/week	M 50	NTP, 1993
37		B6C3F1 Mice	Net increased (46%) incidence of hepatocellular adenoma or carcinoma	gavage 5 times/week	F 50	NTP, 1993
	271	CD-1 mice	Pathology, haematology	diet	F, M 52	Carlton *et al*., 1996
18		F344 rats	Increased (33%) severity grade of nephropathy Net increased (15%) incidence of nephropathy Increased (10%) relative liver weight	gavage 5 times/week	F 50, 10 for liver weight	NTP, 1993
18		F344 rats	Net increased (24%) incidence of liver necrosis Net increased (45%) severity grade of nephropathy Net increased (39%) incidence of forestomach ulcer	gavage 5 times/week	M 50	NTP, 1993
18		F344 rats	Net increased (10%) incidence of kidney adenoma	gavage 5 times/week	M 50	NTP, 1993
16		Sprague-Dawley rats	Increased (15%) absolute liver weight	diet	F 65	Carlton *et al*., 1996
21.4	8.6	Mongrels and beagles dogs	Liver toxicity	Capsules 6/week	M, F 4	Hagan *et al*., 1967
67.5	22.5	Baboon	Liver weight	diet	M 4	Evans *et al*., 1979

**The VKM panel has converted the LOAEL values from the NTP study from 5 times/week to mg/kg bw/day.*

DOSE-RESPONSE MODELLING

The VKM Panel decided to use the benchmark dose (BMD) approach to determine a point of departure for the risk characterisation of coumarin in this opinion. The US Environmental Protection Agency's benchmark dose software (BMDS) version 2.1.2 was used for modelling animal data from the NTP 1993 study (NTP, 1993). Out of the studies summarised in Table 3, the data from this study were chosen because of the high quality of the study and the comprehensively documented results.

The BMD analyses were performed on continuous dose-response data of the 13-week gavage study (effects on plasma cholinesterase levels in rats) and of the 15-month interim evaluation in the 2-year gavage study (effects on absolute and relative liver weight and liver enzymes in rats and on relative liver weight in mice).

For the continuous dose-response data, the VKM Panel chose to calculate BMD_{05} (the estimated dose that represents 5% change in response relative to background predicted by the fitted model) and the correspondent lower 95-confidence bound $BMDL_{05}$ (dose where the response is likely to be smaller than 5%) values as recommended by EFSA, by using the dose-

response models Power, Polynominal, Linear and Hill available in the BMDS Software (EFSA, 2009).

The quantal data from the 2-year gavage study (effects on renal tumour, forestomach ulcers and liver necrosis in rats) were also considered. However, the data was not found suitable for mathematical modelling.

The results of the BMD analysis of the relevant animal data in the NTP study are presented in the following sections.

13-week gavage study

Rats

Reduced production and level of plasma cholinesterase is regarded as an unspecific indicator of hepatotoxicity (inhibited protein synthesis). In females and males, there was a clear dose-related decrease (P<0.0001) in plasma cholinesterase, and acceptable model fit (P>0.1) was seen only with the Polynomial model and Hill model (Table 4). In females, the best fit was demonstrated with the Hill model, which estimated a $BMDL_{05}$ value of 3 mg/kg bw (Table 4 and Figure 2). In males, the Polynomial model indicated a $BMDL_{05}$ value of 8 mg/kg bw. These values refer to gavage dosing 5 times per week.

Table 4. *BMD_{05} and $BMDL_{05}$ values (mg/kg bw, 5 times/week) for plasma cholinesterase levels for male and female rats in the 13-week gavage study (NTP, 1993).*

		Males			Females		
End point	**Test**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**
Plasma cholinesterase**	Power	<0.1	372	120	<0.1	23	20
	Polynomial	0.28	11	8	0.72	5	4
	Linear	<0.1	372	120	<0.1	23	20
	Hill	<0.1	15	7	0.94	7	3

*P-value greater than 0.1 imply that the model chosen seems to adequately describe the data (test 4), according to the US EPA BMDS Software.
**There is a dose-response (P<0.0001) in all the models (test 1).

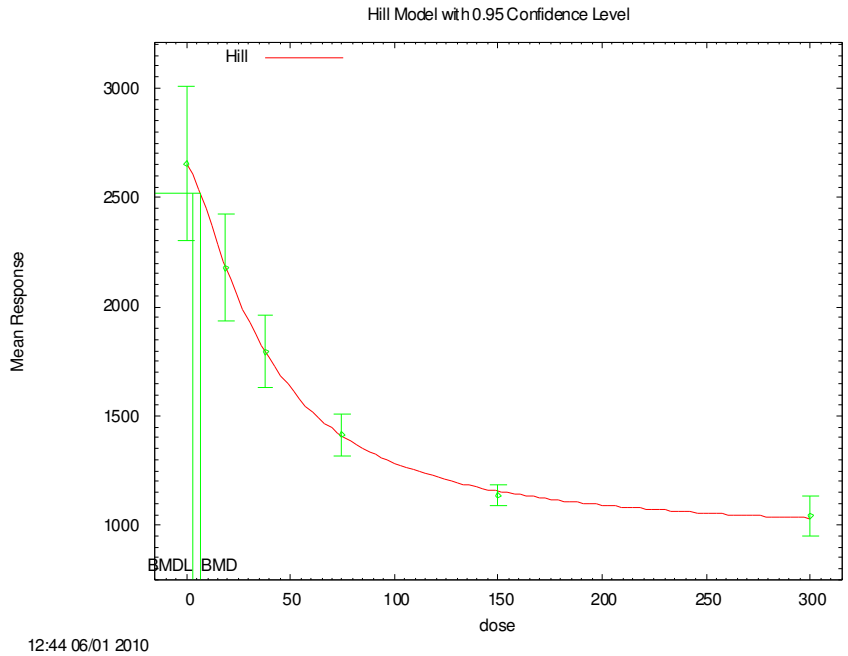

Figure 2. *Plasma cholinesterase in female rats in the 13 week gavage study (NTP, 1993).*

15-month interim evaluation in the 2-year gavage study

Mice

The calculated BMD_{05} and $BMDL_{05}$ values for relative increase in liver weight based on data in the 15-month interim evaluation in mice (NTP, 1993) are presented in Table 5. The data for absolute liver weight did not show dose-response, even though there was an acceptable fit. For relative liver weight, the lowest $BMDL_{05}$ of 16 mg/kg bw in female mice was estimated with low fit to the Hill model. Much better fit was seen with Power and Linear models estimating $BMDL_{05}$ values of approximately 30 mg/kg bw for both sexes. This dose refers to the 5 times a week administration.

Table 5. *BMD_{05} and $BMDL_{05}$ values for relative liver weight in mice (mg/kg bw, 5 times/week) at 15-month interim evaluation (NTP, 1993).*

		Males			Females		
End point	**Test**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**
Relative liver weight**	Power	0.93	123	32	0.30	42	31
	Polynomial	NA***	122	9	NA	15	7
	Linear	0.45	46	27	0.30	42	31
	Hill	NA	104	30	0.12	41	16

**P*-value greater than 0.1 imply that the model chosen seems to adequately describe the data (test 4), according to the US EPA BMDS Software.
**There is a dose-response (test 1) for relative liver weight in males (P=0.002) and females (P<0.0001).
***Not applicable - the test for fit is not valid.

Rats

In rats, dose-response data on liver weight, alkaline phosphatase, alanine aminotransferase, sorbitol dehydrogenase and gamma-glutamyltransferase were modelled. In male rats, the liver enzymes did not show fit with any models, although dose-response was indicated. In females, there was a significant dose-response (P<0.001) as well as acceptable model fit (P>0.29) with the Power model for alkaline phosphatase and alanine aminotransferase, and the $BMDL_{05}$ for these effects were 14 mg/kg bw and 29 mg/kg bw, respectively (data not shown).

The calculations of BMD_{05} and $BMDL_{05}$ for absolute and relative increase in liver weight in rats are presented in Table 6. The lowest $BMDL_{05}$ and highest fit was seen in females with the Linear model (Figure 3) on relative liver weight: BMD_{05} = 11 mg/kg bw, $BMDL_{05}$ = 10 mg/kg bw. These doses refer to the 5 times a week administration.

Table 6. *BMD_{05} and $BMDL_{05}$ values for absolute and relative liver weight in rats (mg/kg bw, 5 times/week) at 15-month interim evaluation (NTP, 1993).*

		Males			Females		
End point	**Test**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**	**Fit***	**BMD_{05}**	**$BMDL_{05}$**
Absolute liver weight**	Power	0.46	31	17	0.68	15	11
	Polynomial	NA***	6	2	NA	7	3
	Linear	0.46	31	17	0.35	15	10
	Hill	0.21	30	<0.1	0.58	10	4
Relative liver weight**	Power	0.41	14	11	0.66	15	10
	Polynomial	NA	8	3	NA	12	5
	Linear	0.72	14	11	0.71	11	10
	Hill	NA	14	14	NA	16	8

**P*-value greater than 0.1 imply that the model chosen seems to adequately describe the data (test 4), according to the US EPA BMDS Software.
**There is a dose-response (P<0.0001) in all the models (test 1).
***Not applicable - the test for fit is not valid.

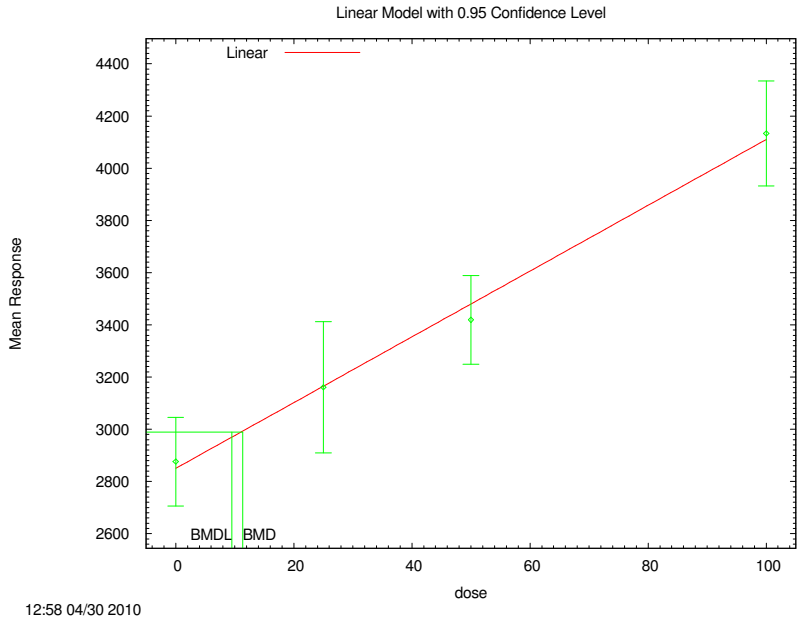

Figure 3. *Relative liver weight in female rats at 15-month interim evaluation (NTP, 1993).*

DERIVATION OF TDI

The most significant hazards of coumarin (as summarised in Table 3) appears to be liver toxicity, which is well documented, and demonstrated in mice, rats, dogs, baboons and humans (NTP, 1993; Carlton *et al.*, 1996; Hagan *et al.*, 1967; Evans *et al.*, 1979, Bergmann, 1999) and kidney adenomas in male rats (NTP, 1993). Liver toxicity in rodents is related to dose-response changes observed in specific liver enzymes, as well as plasma cholinesterase and an unspecific dose-response change in relative liver weight (NTP, 1993).

The NTP 1993 report, which is a comprehensive study of high quality, provides sufficient dose-response data for model simulation and BMD/BMDL calculations and the possible identification of a point of departure for adverse effects of coumarin. Using the BMD approach, the best model fit of the dose-response data combined with the lowest $BMDL_{05}$ was seen for increased relative liver weight in female rats, which gave a $BMDL_{05}$ of 7 mg/kg bw/day (converted from 10 mg/kg bw, 5 times per week, see Table 6 and Figure 3). This $BMDL_{05}$ is approximately 2.5 times below the LOAEL of 18 mg/kg bw/day for kidney adenomas in male rats (NTP, 1993) and close to the identified NOAEL of 8.6 mg/kg bw/day for liver toxicity in dogs (Hagan *et al.*, 1967) as used by EFSA to establish their TDI of 0.1 mg/kg bw/day. The lowest dose causing hepatotoxicity in the most susceptible human individuals was 0.4 mg/kg bw/day (Bergmann, 1999).

For the derivation of a TDI for coumarin, the VKM Panel used the $BMDL_{05}$ for increased relative liver weight in female rats of 7 mg/kg bw/day. The VKM Panel used a standard uncertainty factor of 100 to account for inter- and intraspecies variation and established a TDI for coumarin of 0.07 mg/kg bw/day.

EXPOSURE CHARACTERISATION

OCCURRENCE, SOURCES OF EXPOSURE AND REGULATORY ASPECTS

Coumarin is a naturally occurring flavouring compound present in many plants. The substance can be isolated from e.g. Tonka beans, woodruff and sweet clover and is found in high levels in some essential oils, particularly cassia leaf oil, cinnamon leaf oil, cinnamon bark oil and in lavender oil and peppermint oil (EFSA, 2004; Lake, 1999).

Oral intake of coumarin is mostly related to consumption of cinnamon-containing foods or cinnamon as a spice. This includes both direct addition of cinnamon to foods as well as the use of cinnamon oils and other cinnamon extracts by the food industry (BfR, 2006a).

Coumarin may not be added as such to foodstuffs or to flavourings, but may be present in a foodstuff either naturally or following the addition of flavourings prepared from natural raw materials. The maximum permitted levels of coumarin in foodstuffs are given in Annex II of European Directive 88/388/EEC (EEC, 1988). The general limit for coumarin in food and non-alcoholic beverages is 2 mg/kg. For alcoholic beverages and certain caramel confectionery, the permitted concentration is 10 mg/kg and for chewing gum 50 mg/kg. However, according to Regulation no 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods (EEC, 2008), the European Commission has recently adopted new maximum levels for coumarin in certain compound food. The new restrictions shall apply from 20 January 2011. The new maximum levels are:

Compound food in which the presence of the substance is restricted	***Maximum level***
Traditional and/or seasonal bakery ware containing a reference to cinnamon in the labelling	50 mg/kg
Breakfast cereals including muesli	20 mg/kg
Fine bakery ware, with the exception of traditional and/or seasonal bakery ware containing a reference to cinnamon in the labelling	15 mg/kg
Desserts	5 mg/kg

In cosmetics, synthetic coumarin is added as a fragrance ingredient to perfumes, skin gels, lotions and deodorants (BfR, 2007). It was found in 57% of 73 deodorants on the European market (Rastogi *et al*., 1998). According to the 7th amendment of the Cosmetic Directive (76/768/EEC), labelling of coumarin is required if present in a concentration of 10 mg/kg (ppm) or higher in leave-on or 100 mg/kg in rinse-off products. There is no upper limit to the concentration of coumarin which may be present in finished cosmetic products in the Cosmetic Directive (EEC, 1976). Coumarin has been found in concentrations ranging from 0.5% to 6.4% in fine fragrances and in less than 0.01% in detergents (Floc'h *et al*., 2002).

The German Federal Institute for Risk Assessment (BfR) has also highlighted the possible use of Cassia cinnamon in cinnamon capsules sold as food supplements or as dietetic foods to reduce blood sugar levels in type II Diabetes mellitus as an important source to coumarin exposure (BfR, 2006b).

COUMARIN CONTENT IN CINNAMON

There are several species of cinnamon, and it is worth noting that the content of coumarin could vary considerably both in various parts of the cinnamon tree (bark, leaves, roots) and

between different cinnamon species. The coumarin levels are primarily related to the flavour and content of essential oil in the cinnamon species. As a spice the internal bark of the cinnamon tree is used, in dried form as sticks and in ground form as cinnamon powder.

It is very important to differentiate between the original cinnamon from Sri Lanka (Ceylon cinnamon) and species of Cassia cinnamon (e.g. China cinnamon, Padang cinnamon) as the chemical composition of the two types are different (BfR, 2006a). Ceylon cinnamon contains eugenol and benzyl-benzoate, but in contrast to Cassia cinnamon the levels of coumarin are low. For Ceylon cinnamon coumarin levels from below the detection level to 190 mg/kg (n = 12) have been identified, while the levels in Cassia cinnamon ranged from 700 to a maximum level of 12200 mg/kg (Miller *et al*., 1995 cited in BfR, 2006a). According to the European Spice Association (ESA), the content of coumarin in Cassia cinnamon varies within the same range (from 1500-8000 mg/kg). In the BfR Health Assessment No 043/2006, it is referred to coumarin levels between 2300 and 3300 mg/kg spice in the cinnamon powder analysed (n = 5). The high levels suggest that the analysed powder was Cassia cinnamon (BfR, 2006a).

Analyses of stick cinnamon identified relatively low values of under 15 mg/kg for Ceylon cinnamon (n = 1) and of 90, 290 and 530 mg/kg for three samples of Cassia cinnamon (BfR, 2006a). Cassia cinnamon is currently the main species of cinnamon used in food products on the Norwegian market (personal communication, Toro, Norway).

ANALYTICAL DATA ON THE COUMARIN LEVELS IN CINNAMON-CONTAINING FOODS

The Norwegian Food Safety Authority has in 2008 and 2009 conducted two small surveys to investigate the level of coumarin in some selected food products containing cinnamon sold on the Norwegian market. A total of 28 food products, including both cinnamon bark and cinnamon powder were analysed (see Tables A1 and A2 in Appendix I). The survey from 2009 also included 14 food supplements containing cinnamon (see Table A2 and the section "Exposure to coumarin from food supplements" on page 44 for further information).

An overview of the average coumarin levels in different food categories is shown in Table 7. The results show that the average coumarin levels in ginger bread, cinnamon buns and similar bakery products and cinnamon-containing cakes were 16.4, 18.0 and 22.5 mg/kg, respectively. The highest coumarin level in the survey was found in a sample of cinnamon buns at 43.9 mg/kg. The average coumarin content from 6 samples of thin pastry with cinnamon ("smurte lefser") was 6.8 mg/kg. Coumarin was not detected in mulled wine ("Tomtegløgg") or fruit tea with cinnamon. A special brand of tea (Yogi tea) with a high content of cinnamon was included in the survey conducted in 2009. The average coumarin level in the two samples (ready to drink tea) was 16.2 mg/kg. Of the two different cinnamon powders analysed, the organic variant had the highest coumarin content at 3330 mg/kg. The highest coumarin level of 4070 mg/kg was found in an organic cinnamon bark. It should be noted that the number of analysed samples in the two surveys was limited.

Breakfast cereals with cinnamon are commonly consumed in many countries and could therefore contribute to the total coumarin intake from food. However, this food category has not been included since there are very few such products sold on the Norwegian market.

Table 7. *Coumarin levels in food products containing cinnamon sold on the Norwegian market.*

Food category	Number of samples	Mean level (mg/kg)	Max. level (mg/kg)
Ginger bread	4	16.4	22.9
Cinnamon buns and similar bakery products	6	18.0	43.9
Cinnamon-containing cakes	2	22.5	40.2
Thin pastry with cinnamon	6	6.8	20.1
Mulled wine/ Glühwein	2	< 1 (n.d.)[a]	< 1 (n.d.)
Fruit tea with cinnamon	1	< 1 (n.d.)	< 1 (n.d.)
Cinnamon-based tea[b]	2	16.2[c]	22.7[c]
Cinnamon stick	1	49.9	49.9
Cinnamon powder	1	2350	2350
Cinnamon powder (organic)	1	3330	3330
Cinnamon bark[b] (organic)	1	4070	4070
Cinnamon bark (crushed)[b]	1	37.9	37.9

[a]n.d. = not detected.
[b]Samples measured in the survey conducted in 2009.
[c]Ready to drink tea.

EXPOSURE TO COUMARIN FROM FOOD

There are many limitations in the intake calculations for coumarin from the different food and drink categories presented in this opinion, due to a limited sample size of analysed products and a lack of consumption data for the specific food items that contain cinnamon. Therefore, many assumptions had to be done when the intake was calculated. Taking these limitations into account, the VKM Panel 4 has considered that the exposure to coumarin in the Norwegian population could be best presented in the following way:

- Methodological descriptions
- Assumptions in the intake calculations
- Total coumarin intake from cinnamon-containing food and drinks based on data in the national food consumption surveys (intake from porridge not included)
- Coumarin intake from cinnamon powder sprinkled on oatmeal porridge and rice porridge
- Assumed worst intake scenarios of coumarin from different cinnamon-containing food categories, such as:
    - Cinnamon-based tea
    - Ginger bread
    - Cinnamon buns and similar bakery products
    - Thin pastry with cinnamon
    - Cinnamon-containing cake
- Results from a research project on the total intake of cinnamon in the Norwegian diet
- Summary of the intake calculations

Methodological descriptions

In the present opinion, the intake calculations for coumarin from food and drinks are based on both data from the nationally representative food consumption surveys Norkost, Ungkost, Småbarnskost and Spedkost, as well as on assumed worst intake scenarios from different cinnamon-containing food products. A short description of the four consumption surveys is given here:

- **Adults;** Norkost 1997 (Johansson and Solvoll, 1999), Norkost is based on a quantitative food frequency questionnaire (FFQ) that was answered by 1291 males and 1381 females aged 16-79 years.
- **13-year-old adolescents;** Ungkost 2000 (Øverby and Frost Andersen, 2002). Ungkost 2000 is based on a 4-day food consumption registration (1005 adolescents), where portions should be assigned according to an illustrative book with different food portion sizes.
- **9-year-old children;** Ungkost 2000 (Øverby and Frost Andersen, 2002). Ungkost 2000 is based on a 4-day food consumption registration (815 children), where portions should be assigned according to an illustrative book with different food portion sizes.
- **4-year-old children;** Ungkost 2000 (Pollestad *et al*., 2002). Ungkost 2000 is based on a 4-day food consumption registration (391 children), where portions should be assigned according to an illustrative book with different food portion sizes.
- **2-year-old children;** Småbarnskost (2007) (Kristiansen *et al*. 2009). Småbarnskost is based on a semi-quantitative FFQ answered by 1674 participating children (829 males, 826 females and 19 children of unspecified gender).
- **1-year-old children;** Spedkost (2006/07) (Øverby *et al*., 2009). Spedkost is based on a semi-quantitative FFQ answered by 1635 participating children (807 males, 826 females and 2 children of unspecified gender).

Additionally, results from a research project on the total intake of cinnamon in the Norwegian diet, which recently has been conducted at the University of Oslo, have been included as a basic for comparison. A short description of the total intake study is given here:

- **18-80-year olds;** Spices and herbs in the Norwegian diet (M.H. Carlsen, L.F. Andersen and R. Blomhoff, manuscript in preparation). The exposure to different spices, including cinnamon, was calculated by using two different dietary methods; semi-quantitative FFQ (*n*=344) and 28-day dietary registration (*n*=146).

Consumers only

The coumarin intake calculated from the data in the consumption surveys is based on only the persons who actually had a consumption of cinnamon-containing products or similar products as described above (consumers only).

Body weight

The mean body weights reported for the different age groups in the consumption surveys have been used to calculate the intake in mg coumarin/kg body weight for an easy comparison with the TDI. An overview of these body weights is shown in Table 8. For adults, a default body weight of 60 kg has been used as this is customary for establishment of a TDI. It should be noted that the mean body weight for Norwegian adults reported in the consumption survey Norkost was higher than 60 kg for both men (≈ 80 kg) and women (≈ 66 kg) (Johansson & Solvoll, 1999).

Table 8. *Mean body weight of children, adolescents and adults in Norway.*

Age	Mean body weight (kg)
1-year-olds	9.9 kg
2-year-olds	12.8 kg
4-year-olds	18 kg
9-year-olds	32 kg
13-year-olds	49.5 kg
Adults	60 kg*

* Default value.

Assumptions in the intake calculations

Due to the limitations in the consumption data for specific food items containing cinnamon it has been necessary to introduce some assumptions in the intake calculations which would influence the reliability of the results. Some assumptions could lead to an overestimation of the intake, while others could result in an underestimation. A description of the most important assumptions in the intake calculations for each food and drink category is described below. It is important to note that each step in the dietary exposure assessment is affected by scientific uncertainties and will contribute to the overall uncertainty of the risk estimates when the results of the total calculated coumarin intake is interpreted (see "Uncertainty and variability" on page 53 for more information).

Cinnamon-based tea

None of the Norwegian consumption surveys include data on the consumption of cinnamon-based tea as such. The coumarin intake from cinnamon-based tea is therefore based on the assumption that all available data on tea consumption in the consumption surveys is replaced by similar amounts of cinnamon-based tea. Consumption of tea is not considered likely for 1- and 2-year-old children.

Ginger bread

None of the Norwegian consumption surveys include data on the consumption of ginger bread as such. The calculations are therefore based on the reported consumption data for biscuits, ginger nuts and other similar products. It is assumed that consumers substitute their usual consumption of these products with ginger bread in the period around Christmas.

Cinnamon buns and similar bakery products

Cinnamon buns as such are not specified in any of the FFQs used in the Norwegian consumption surveys. The coumarin intake from this food category is therefore based on the reported consumption of similar bakery products.

Thin pastry with cinnamon

Only the consumption survey Ungkost has data on the consumption of thin pastry with cinnamon. It has therefore not been possible to calculate the coumarin intake from this food category for small children and adults.

Cinnamon-containing cakes

Cinnamon-containing cakes are not specified in any of the FFQs used in the Norwegian consumption surveys. The coumarin intake in this food category is therefore based on the reported consumption of similar cakes and that consumers substitute their usual consumption of these products with cinnamon-containing cakes in the period around Christmas. There is no data available for the consumption of cakes among 1- and 2-year-old children, so it has not been possible to calculate the coumarin intake for these age groups.

Total coumarin intake from cinnamon-containing food and drinks based on data in the national food consumption surveys (intake from porridge not included)

The total coumarin intake from cinnamon-containing food products in different age groups is shown in Table 9a. The food categories included are ginger bread, cinnamon buns and similar bakery products, thin pastry with cinnamon and cinnamon-containing cakes. Consumption estimates for each relevant food in the dietary surveys were multiplied with the corresponding average coumarin levels presented in Table 7 and totalled for each individual. The calculations are based on only the participants in the dietary surveys who actually reported a consumption of relevant food products (consumers only).

Table 9a. *Total coumarin intake from cinnamon-containing food among consumers only in different age groups (intake from tea and porridge not included).*

Total coumarin intake from food (mg/kg bw/day)[a]			
Age groups	**N (%)[b]**	**Mean**	**95 percentile**
1-year-olds	1107 (68%)	0.006	0.02
2-year-olds	1460 (87%)	0.009	0.02
4-year-olds	277 (71%)	0.02	0.06
9-year-olds	475 (59%)	0.02	0.05
13-year-olds	449 (45%)	0.01	0.04
Adults	2175 (81%)	0.004	0.01

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).

The results show that the mean total intakes of coumarin from food range from 0.004 mg/kg bw/day in adults to 0.02 mg/kg bw/day in 4-and 9-year-old children. The intake at the 95 percentile was found to be highest in 4- and 9-year-old children (0.05-0.06 mg/kg bw/day).

Table 9b shows the total coumarin intake in different age groups when also the consumption of cinnamon-based tea is included in the calculations. The VKM Panel 4 has considered it appropriate to present the data which include the intake from cinnamon-based tea in a separate table, as consumption of such tea probably is not very common in the overall population.

Table 9b. *Total coumarin intake from cinnamon-containing food and cinnamon-based tea among consumers only in different age groups (intake from porridge not included).*

Total coumarin intake from food (mg/kg bw/day)[a]			
Age groups	**N (%)[b]**	**Mean**	**95 percentile**
1-year-olds[c]	1107 (68%)	0.006	0.02
2-year-olds[c]	1460 (87%)	0.009	0.02
4-year-olds	284 (73%)	0.02	**0.07**
9-year-olds	508 (63%)	0.02	**0.07**
13-year-olds	542 (54%)	0.02	0.06
Adults	2456 (92%)	0.05	**0.2**

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).
[c]Intake from cinnamon-based tea not considered likely.

The results show that the consumption of cinnamon-based tea leads to a significant increase in the mean and high total coumarin intake among adults compared to the data in Table 9a.

Adolescents (13-year-olds) will also have a small increase in both the mean and high total coumarin intake, whereas only the high intake (95 percentile) among 4- and 9-year-old children is increased because of the intake of cinnamon-based tea. Consumption of cinnamon-based tea is not considered likely for 1- and 2-year-olds.

The mean and high (95 percentile) consumption of the different food and drink categories included in the total intake of coumarin for all age groups are shown in Tables A3-A7 in Appendix II. These tables also show the mean and high (95 percentile) coumarin intakes calculated for the separate food categories.

Coumarin intake from cinnamon powder sprinkled on oatmeal porridge and rice porridge

It should be noted that intake from cinnamon powder sprinkled on oatmeal porridge or rice porridge is not included in the calculated total intake from the consumption surveys shown in Tables 9a and 9b. This could be an important coumarin source from food, depending on the amount and frequency of cinnamon powder consumed.

Rice porridge with cinnamon and sugar is a traditional Norwegian meal which is commonly consumed by the whole population. Different variants of oatmeal porridge are a very common meal for small children in Norway. It is not known if it is equally common to use cinnamon and sugar on oatmeal porridge as on rice porridge, but such use has been reported, even for 1- and 2-year-old children.

The intake scenarios for coumarin exposure from cinnamon powder sprinkled on oatmeal porridge and rice porridge in this opinion have been based on the amount of cinnamon powder used and the frequency of consumption reported in the food consumption surveys for the different age groups. The frequency of consumption (mean and 95 percentile) of oatmeal porridge and rice porridge for 1- and 2-year-old children and adults is shown in Table A8 in Appendix III.

It has not been possible to provide exact data on the frequency of consumption of oatmeal porridge and rice porridge for 4-, 9- and 13-year-olds as the food consumption survey for these age groups (Ungkost 2000) is based on a 4-day food consumption registration. The intake of coumarin has therefore been calculated on the basis of the assumed frequencies shown in Table A9 in Appendix III.

An overview of the different intake scenarios for the exposure to coumarin through consumption of cinnamon powder sprinkled on oatmeal porridge or rice porridge in the different age groups, taking into account the frequency of consumption, is illustrated in Tables 10 and 11.

The intake scenarios are based on the following assumptions:

- The coumarin level in cinnamon powder is 3000 mg/kg (Abraham *et al.*, 2010).
- One teaspoon (ts) of cinnamon weighs 2.69 g (M.H. Carlsen, L.F. Andersen and R. Blomhoff, manuscript in preparation).
- Amounts of ¼ - 1 ts of cinnamon powder sprinkled on both types of porridge for children up to 4 years.
- Amounts of ½ - 2 ts of cinnamon powder sprinkled on both types of porridge for 9-year-olds, adolescents and adults.

The intake calculations are illustrated by an example:

It is assumed that a ½ teaspoon of cinnamon is sprinkled on one portion of oatmeal porridge for 1- and 2-year-old children. This would correspond to an intake of 3.0 mg/g x 1.35 g = 4.1 mg coumarin/meal. Taking into account a consumption of oatmeal porridge 4.2 times a week for 1-year-olds, the coumarin intake would be 17.2 mg/week or 2.5 mg/day, equivalent to 0.25 mg/kg bw/day. The 2-year-olds consume oatmeal porridge 3.1 times a week, which would result in a coumarin intake of 12.7 mg/week or 1.8 mg/day, equivalent to 0.14 mg/kg bw/day.

Table 10. *Coumarin intake based on exposure scenarios for different amounts of cinnamon powder sprinkled on oatmeal porridge. The calculations are based on both mean and a high frequency of consumption reported in the National consumption surveys.*

	Coumarin intake based on mean frequency of consumption (mg/kg bw/day)				**Coumarin intake based on a high frequency of consumption (mg/kg bw/day)**			
	Amount of cinnamon				**Amount of cinnamon**			
Age groups	**¼ ts**	**½ ts**	**1 ts**	**2 ts**	**¼ ts**	**½ ts**	**1 ts**	**2 ts**
1-year-olds	**0.12**	**0.25**	**0.49**	-	**0.41**	**0.82**	**1.63**	-
2-year-olds	**0.07**	**0.14**	**0.28**	-	**0.16**	**0.32**	**0.63**	-
4-year-olds	0.03	0.06	**0.13**	-	**0.08**	**0.16**	**0.32**	-
9-year-olds	-	0.02	0.04	**0.07**	-	0.05	**0.11**	**0.22**
13-year-olds	-	0.006	0.01	0.02	-	0.03	**0.07**	**0.14**
Adults	-	0.01	0.02	0.04	-	0.04	**0.09**	**0.17**

Cinnamon powder sprinkled on oatmeal porridge will result in high intakes of coumarin for 1-year-old children in all the intake scenarios shown in Table 10. The scenario that is based on a mean frequency of consumption and 1 ts of cinnamon powder sprinkled on the porridge results in a coumarin intake of 0.49 mg/kg bw/day, whereas the worst intake scenario (high frequency) based on the similar assumptions gives a intake of 1.63 mg/kg bw/day.

Both 2-year-olds and 4-year-olds will also have high coumarin intakes based on a mean frequency of consumption of oatmeal porridge, whereas all age groups were found to have high coumarin intakes with a high frequency of consumption.

Table 11. *Coumarin intake based on exposure scenarios for different amounts of cinnamon powder sprinkled on rice porridge. The calculations are based on both mean and high percentile frequency of consumption reported in the National consumption surveys.*

	Coumarin intake based on mean frequency of consumption (mg/kg bw/day)				**Coumarin intake based on high frequency of consumption (mg/kg bw/day)**			
	Amount of cinnamon				**Amount of cinnamon**			
Age groups	**¼ ts**	**½ ts**	**1 ts**	**2 ts**	**¼ ts**	**½ ts**	**1 ts**	**2 ts**
1-year-olds	0.01	0.03	0.06	-	0.03	0.06	**0.11**	-
2-year-olds	0.01	0.03	0.05	-	0.02	0.04	**0.09**	-
4-year-olds	0.007	0.01	0.03	-	0.01	0.03	0.06	-
9-year-olds	-	0.008	0.02	0.03	-	0.01	0.03	0.05
13-year-olds	-	0.005	0.01	0.02	-	0.008	0.02	0.03
Adults	-	0.004	0.008	0.02	-	0.009	0.02	0.03

The frequency of consumption of rice porridge is much less than for oatmeal porridge, but the contribution from cinnamon powder sprinkled on rice porridge will nevertheless be an important contribution to the intake of coumarin in all age groups. This is especially valid for the worst intake scenarios based on a high frequency of consumption and the largest amount

of cinnamon powder used. In this scenario, the coumarin intake was 0.11 and 0.09 mg/kg bw/day for 1-year-olds and 2-year-olds, respectively (Table 11).

The intake scenarios presented above are representative only for consumers with a registered consumption of either oatmeal porridge (Table 10) or rice porridge (Table 11) in the national consumption surveys. The VKM Panel 4 has also looked into the situation if consumption of both oatmeal porridge and rice porridge in the same consumers were taken into account in the intake scenarios. Table A8 in Appendix shows the number of consumers and frequency of consumption (mean and 95 percentile) among consumers with a consumption of both oatmeal porridge and rice porridge in the age groups 1-year-olds, 2-year-olds and adults.

The results show that there is only an insignificant reduction in the mean frequencies of consumption for this selection of consumers in the three age groups, whereas the high frequencies of consumption (95 percentile) are unchanged compared to consumers of only oatmeal porridge. The frequency data for the consumption of rice porridge are more or less identical for the two selections of consumers. It can therefore be concluded that the coumarin intakes for consumers with a consumption of both oatmeal porridge and rice porridge would be of the same order of magnitude as the intake scenarios presented in Table 10 and 11.

Assumed worst intake scenarios of coumarin from different cinnamon-containing food categories

The intake calculations that are based on information from the national food consumption surveys are influenced by uncertainties due to limitations in the consumption data for specific cinnamon-containing foods. To get an additional picture of the coumarin intake among individuals with a high consumption of cinnamon-containing foods and drinks, the VKM Panel 4 also decided to perform some assumed worst intake scenarios for the different food categories included in the total coumarin intake shown in Tables 9a and 9b. The maximum coumarin levels for the different food categories shown in Table 7 have been used to estimate the intake of coumarin in the assumed worst case scenarios.

The high (95 percentile) consumption of coumarin from the different food and drink categories has also been calculated for all age groups in the food consumption surveys (see Tables A3-A7 in Appendix II). It should be noted that the assumed worst intake scenarios shown below are not directly comparable to the results shown in Tables A3-A7 due to differences in the coumarin levels that are used in the two calculations.

Worst intake scenario of coumarin from cinnamon-based tea

Table 12 shows an assumed worst intake scenario of coumarin from cinnamon-based tea for different age groups in the Norwegian population. The maximum coumarin level in Yogi tea was measured to 22.7 mg/kg (see Table 7 and Table A1 in Appendix I). Yogi tea is a special type of tea with a blend of different ingredients and aromas such as e.g. ginger, cardamom, cinnamon and cloves. The product Yogi tea Classic is marketed as a tea with an especially high content of cinnamon (55-61%).

As a worst case scenario, it is assumed that adults who prefer cinnamon tea could consume up to four cups (size 0.25 litre) of cinnamon-based tea daily. Smaller frequencies of consumption were assumed for the other age groups. Consumption of tea was not considered likely for 1- and 2-year-old children.

Table 12. *Assumed worst intake scenarios of coumarin from cinnamon-based tea.*

Age groups	Mean bw (kg)	Amount consumed (kg)	Frequency (times/day)	Coumarin intake (mg/kg bw/day)[a]
4-year-olds	18	0.25	1	**0.3**
9-year-olds	32	0.25	1	**0.2**
13-year-olds	49.5	0.25	2	**0.2**
Adults	60[b]	0.25	4	**0.4**

[a]Assumed intake based on the maximum level of 22.7 mg coumarin/kg found in Yogi tea (see Table 7).
[b]For adults, a default body weight of 60 kg was used.

Adults, who are assumed to have the highest consumption of tea among the age groups, were found to have a daily intake of 0.4 mg coumarin/kg bw by consuming four cups of cinnamon-based tea. The coumarin intakes from cinnamon-based tea in the other age groups ranged from 0.2-0.3 mg/kg bw/day.

Worst intake scenario of coumarin from ginger bread

Table 13 shows an assumed worst intake scenario of coumarin from ginger bread for different age groups in the Norwegian population. The estimated intake is based on the measured maximum level of 22.9 mg coumarin/kg found in ginger bread (see Table 7 and Table A1 in Appendix I). Ginger bread is considered as a seasonal product that is consumed before, during and after Christmas. A consumption of 100 g ginger bread (approximately 20 pieces) three times a week is assumed in this period of the year for the age groups 4-, 9- and 13-year-olds and adults. A consumption of 50 g ginger bread (approximately 10 pieces) once a week in the Christmas period is assumed for 1- and 2-year-old children.

Table 13. *Assumed worst intake scenarios of coumarin from ginger bread*

Age groups	Mean bw (kg)	Amount consumed (kg)	Frequency (times/week)	Coumarin intake (mg/kg bw/day)[a]
1-year-olds	9.9	0.05	1	0.02
2-year-olds	12.8	0.05	1	0.01
4-year-olds	18	0.1	3	0.05
9-year-olds	32	0.1	3	0.03
13-year-olds	49.5	0.1	3	0.02
Adults	60[b]	0.1	3	0.02

[a]Assumed intake based on the maximum level of 22.9 mg coumarin/kg found in ginger bread (see Table 7).
[b]For adults, a default body weight of 60 kg was used.

The results show that 4-year-old children will have the highest coumarin intake from ginger bread per kg body weight (0.05 mg/kg bw/day).

Worst intake scenario of coumarin from cinnamon buns and similar bakery products

Table 14 shows an assumed worst intake scenario of coumarin from cinnamon buns and similar bakery products for different age groups in the Norwegian population. The maximum coumarin level in cinnamon buns was measured to 43.9 mg/kg (see Table 7 and Table A1 in Appendix I). However, another product called "Pågens kanelgifler" is considered as a more popular brand on the Norwegian market and the assumed worst case scenario are therefore based on high consumption of these cinnamon buns. The coumarin level in this product was measured to 22.6 mg/kg (see Table A1 in Appendix I).

Consumption of cinnamon buns among adolescents is assumed to be quite common. In the worst case scenarios, it is therefore assumed that 13-year-olds could consume one bag of cinnamon buns three times a week, whereas 9-year-olds and adults could consume one bag twice a week. One bag of "Pågens kanelgifler" weighs 260 g and contains 12 pieces. For the

age groups of smaller children different amounts and frequencies of consumption were considered more likely (see Table 14).

Table 14. *Assumed worst intake scenarios of coumarin from cinnamon buns and similar bakery products.*

Age groups	Mean bw (kg)	Amount consumed (kg)	Frequency (times/week)	Coumarin intake (mg/kg bw/day)[a]
1-year-olds	9.9	0.13	1	0.04
2-year-olds	12.8	0.13	1	0.03
4-year-olds	18	0.26	1	0.05
9-year-olds	32	0.26	2	0.05
13-year-olds	49.5	0.26	3	0.05
Adults	60[b]	0.26	2	0.03

[a]Assumed intake based on the level of 22.6 mg coumarin/kg found in cinnamon buns ("Pågens kanelgifler") (see Table A1 in Appendix 1).
[b]For adults, a default body weight of 60 kg was used.

4-, 9- and 13-year-olds were all found to have an intake of 0.05 mg coumarin/kg bw/day from cinnamon buns and similar bakery products, taking into account the assumptions of amounts and frequencies of consumption shown in Table 14.

Worst intake scenario of coumarin from thin pastry with cinnamon

Table 15 shows an assumed worst intake scenario of coumarin from thin pastry with cinnamon for different age groups in the Norwegian population. The estimated intake is based on the measured maximum level of 20.1 mg coumarin/kg found in thin pastry with cinnamon (see Table 7 and Table A1 in Appendix I).

Consumption of thin pastry with cinnamon is assumed to be a common meal among adolescents and adults. In the worst case scenarios, it is therefore assumed that 13-year-olds and adults could consume one package of thin pastry with cinnamon three and two times a week, respectively. One package of "Berthas smurte lefser med kanel og sukker" weighs 230 g and contains 2 pieces. For the other age groups, it is assumed a consumption of one package once a week for 9-year-olds and lesser amounts once a week for the smaller children.

Table 15. *Assumed worst intake scenarios of coumarin from thin pastry with cinnamon.*

Age groups	Mean bw (kg)	Amount consumed (kg)	Frequency (times/week)	Coumarin intake (mg/kg bw/day)[a]
1-year-olds	9.9	0.05	1	0.01
2-year-olds	12.8	0.05	1	0.01
4-year-olds	18	0.12	1	0.02
9-year-olds	32	0.23	1	0.02
13-year-olds	49.5	0.23	3	0.04
Adults	60[b]	0.23	2	0.02

[a]Assumed intake based on the maximum level of 20.1 mg coumarin/kg found in thin pastry with cinnamon (see Table 7).
[b]For adults, a default body weight of 60 kg was used.

Adolescents (13-year-olds) have the highest intake of coumarin of 0.04 mg/kg bw/day from thin pastry with cinnamon, taking into account the assumptions of amounts and frequencies of consumption shown in Table 15.

Worst intake scenario of coumarin from cinnamon-containing cakes

Table 16 shows an assumed worst intake scenario of coumarin from cinnamon-containing cakes for different age groups in the Norwegian population. The maximum coumarin level in cinnamon-containing cakes was measured to 40.2 mg/kg (see Table 7 and Table A1 in

Appendix I). Cinnamon-containing cakes are considered as a seasonal product that is consumed before, during and after Christmas. According to data from the USDA National Nutrient Database for Standard Reference, 1 piece of cinnamon-containing cake weighs 63 g (USDA, 2004). It is assumed that 13-year-olds and adults could consume 3 pieces of cinnamon-containing cake three times a week in the Christmas period. For the other age groups, it is assumed a consumption of two pieces twice a week for 9-year-olds and lesser amounts once a week for the smaller children.

Table 16. *Assumed worst intake scenarios of coumarin from cinnamon-containing cakes.*

Age groups	Mean bw (kg)	Amount consumed (kg)	Frequency (times/week)	Coumarin intake (mg/kg bw/day)[a]
1-year-olds	9.9	0.03	1	0.02
2-year-olds	12.8	0.03	1	0.01
4-year-olds	18	0.06	1	0.02
9-year-olds	32	0.13	2	0.05
13-year-olds	49.5	0.19	3	**0.07**
Adults	60[b]	0.19	3	0.05

[a]Assumed intake based on the max level of 40.2 mg coumarin/kg found in cinnamon-containing cakes (see Table 7).
[b]For adults, a default body weight of 60 kg was used.

The results show that 13-year-olds could have an intake of 0.07 mg coumarin/kg bw/day from cinnamon-containing cakes. Both 9-year-old children and adults could have coumarin intakes of 0.05 mg/kg bw/day, taking into account the assumptions shown in Table 16.

Results from a research project on the total intake of cinnamon in the Norwegian diet

A new study on the total intake of spices and herbs in the Norwegian diet has recently been conducted at the University of Oslo (M.H. Carlsen, L.F. Andersen and R. Blomhoff, manuscript in preparation). The study has been performed among adults aged 18-80 years, living in the Oslo and Akershus area. The exposure to different spices, including cinnamon, was calculated by two different dietary methods (FFQ and 28-day dietary registration). The number of participants using the FFQ was 344, while 146 of these participants were randomly chosen to register all their consumption of spices and herbs in 28 days.

All amounts of spices used when preparing food at home, such as cinnamon powder in bakery products, sprinkled on porridge, on coffee etc., was registered in the study. The intake of cinnamon from processed food was not included. It should be noted that the dietary registration was conducted from September to March, so no exposure to spices was registered in the summer months. However, it is assumed that this will have little influence on the data for the consumption of cinnamon. The overall results of the exposure to cinnamon calculated in the study are shown in Table 17.

Table 17. *Frequency (times used per month) and amount of cinnamon (g) used each time among Norwegian adults aged 18-80 years.*

Cinnamon	% subjects registered use	Frequency, times used per month		Amount used per time (g)	
		Median	Max	Median	Max
FFQ (*n*=344)	82	2	32+	0.7	8.1
28-day dietary registration (*n*=146)	64	2	24	2.18	16

The data show that the frequencies are quite similar by using the two different dietary methods, whereas the amount of cinnamon used each time is higher in the 28-day dietary registration compared to the FFQ. The explanation for these differences is not known, but the authors suggest that it could be related to difficulties registering the correct amount of spices in a FFQ. Information on whether the same persons have a high intake of cinnamon and a high frequency of consumption is not yet considered.

Calculations in this study also show that the consumption of rice porridge correlated with the consumption of cinnamon (Spearman's rank correlation coefficient 0.49 (FFQ) and 0.45 (28-day dietary registration), whereas no such correlation was found between the consumption of oatmeal porridge and consumption of cinnamon (Spearman's rank correlation coefficient 0.27 (FFQ) and 0.04 (28-day dietary registration).

Coumarin intake in adults based on the results in the total intake study of cinnamon

The mean value of 3000 mg coumarin/kg cinnamon powder (Abraham *et al.*, 2010) could be used to calculate the total coumarin intake based on the frequency and amounts of cinnamon consumption reported in the recent study conducted at the University of Oslo (see Table 18).

The calculated coumarin intake based on a maximum amount and maximum frequency of cinnamon consumption reported in the 28-day dietary registration is shown as a worst case:

3.0 mg/g x 16g x 24/30 = 38.4 mg/day, equivalent to 0.64 mg/kg bw/day in adults with a body weight of 60 kg.

Table 18. *Calculated intake of coumarin (mg/kg bw/day) in Norwegian adults based on median and maximum values for the frequency and amount of cinnamon consumed.*

	Med. freq. and med. amount	**Med. freq. and max. amount**	**Max. freq. and med. amount**	**Max. freq. and max. amount**
Coumarin intake[a] (FFQ)	0.002	0.03	0.04	**0.43**
Coumarin intake[a] (28-d reg.)	0.007	0.05	**0.09**	**0.64**

[a]Calculation based on a coumarin level of 3000 mg/kg in cinnamon powder and a default body weight of 60 kg for adults.

The calculated coumarin intakes based on a median amount and frequency of cinnamon consumption from the two different dietary methods were 0.002 and 0.007 mg/kg bw/day. When using the maximum amount and frequency of cinnamon consumption in the calculation, the coumarin intakes were 0.43 and 0.64 mg/kg bw/day.

EXPOSURE TO COUMARIN FROM FOOD SUPPLEMENTS

The coumarin levels in 14 food supplements (cinnamon capsules or tablets) sold on the Norwegian market have been measured in a survey conducted by the Norwegian Food Safety Authority in 2009/2010. Cinnamon capsules/tablets are claimed to reduce blood sugar levels and are therefore especially marketed to persons with type II Diabetes mellitus. The analysed products were collected in different health food stores in Oslo. Based on information on the average weight per capsule/tablet, the recommended daily dose and the coumarin levels found in the products, the coumarin intake at the recommended dose could be estimated. These

results are presented in Table 19 together with values for the exhaustion of the TDI of 0.07 mg/kg bw/day at the recommended daily dose for persons with a body weight of 60 kg.

Table 19. *Estimated coumarin intake from cinnamon capsules sold on the Norwegian market.*

Product	Average weight per capsule (C)/ tablet (T) (g)	Recommended daily dose (capsules/ tablets)	Coumarin level (mg/kg)	Coumarin intake at recommended dose (mg/day)	Exhaustion of TDI at 60 kg/bw
Lamberts Cinnamon	1.2298 (T)	1	371	0.46	11%
Lamberts Multi-Guard Control	1.4477 (T)	2	155	0.45	11%
NutriFood Kanel	0.5264 (C)	3-4	607	1.12	27%
Nature's Way Cinnamon Standardized	0.6032 (C)	2	2320	2.80	67%
Salus Momordica	1.2526 (T)	4	4.75	0.02	0.5%
Gevita Kanel	0.4608 (C)	1	<1	0.0005	0.01%
Swanson Cinnamon Extract	0.3968 (C)	1	613	0.04	1%
DFI Kanel+	0.6832 (T)	3	235	0.48	11%
Solaray Cinnamon Bark	0.5786 (C)	2	924	1.07	25%
Swanson Cinnamon Gymnema Mulberry Complex	0.9184 (C)	2	3250	5.97	142%
Swanson Full Spectrum Cinnamon	0.4599 (C)	6	2180	6.02	143%
Swanson Cinnulin PF	0.5309 (C)	3	804	1.28	30%
Bio Insu Complex Kanelbarkekstrakt	0.5776 (C)	3	146	0.25	6%
New Nordic DIDA Kanelolje	1.0963 (T)	3	<10	<0.03	<0.7%

High coumarin levels between 2180 and 3250 mg/kg capsule content were measured for three of the cinnamon products. These high coumarin levels indicate that the manufacturers use Cassia cinnamon in the production of the cinnamon capsules. The overall results from all the analysed food supplements in the Norwegian survey showed coumarin intakes in the same order of magnitude as was found in a similar investigation described in a health assessment from BfR in 2006 (BfR, 2006b).

In their study, BfR also looked into high daily intakes of cinnamon from cinnamon capsules sold as food supplements or as dietetic foods to reduce blood sugar levels. The coumarin levels in ten cinnamon products, six marketed as dietetic foods and four as food supplements, were measured by German control authorities. Levels between 2300 to 3300 mg coumarin/kg capsule content were found in products where cinnamon powder had been used in the production. Intake of capsules at the recommended daily dose for a body weight of 70 kg resulted in an exhaustion of between 31 and 64% of the TDI for the six products based on cinnamon powder.

BfR concluded that, since it cannot be assumed that there are different exposure scenarios for diabetics, an additional coumarin exposure through consumption of cinnamon capsules with the abovementioned coumarin levels could lead, in isolated cases, to an exceedance of the TDI of 0.1 mg coumarin/kg bw/day established by EFSA.

EXPOSURE TO COUMARIN FROM COSMETICS

Exposure to coumarin from cosmetic products through the dermal route is another important source that has to be taken into account when the total exposure to the substance is estimated. Synthetic produced coumarin is added as fragrance to cosmetics, such as perfumes, shower gels, body lotions, deodorants and oils (BfR, 2007). There are no maximum limits for coumarin in cosmetics, but according to the 7th amendment of the Cosmetics Directive it must be labelled as an ingredient from specific concentrations upwards (see page 32). Based on the production volume of synthetic coumarin converted to a per capita proportion in the population, dermal exposure could be of significance (BfR, 2006). An average daily coumarin amount of 1.2 mg per US American from an annual production of 113.4 tons in the USA for a population of 250 million has been calculated by Yourick and Bronaugh (1997).

The data on coumarin levels in cosmetics are scarce, and no data are available from products sold on the Norwegian market. Thus, the dermal exposure to coumarin described in this opinion is based on data discussed in Lake (1999) and BfR (2006a, 2007).

Coumarin has been found at concentrations between 0.002 and 0.61% in 71% of US American products examined (Harris and Wisneki, 2001). Lake (1999) has estimated a daily intake of 2.3 mg coumarin (0.04 mg/kg bw for an adult weighing 60 kg), which he considered realistic for a worst case scenario. In this calculation, it is taken into account that the dermal absorption of coumarin is approximately 60% (Huntington Life Sciences, 1996a cited in Lake 1999; Ford *et al*., 2001; BfR, 2006a). Another assumption is that a typical consumer uses only one product at a time containing the 97.5 percentile of coumarin levels in a fragrance mixtures used by several different fragrance manufacturers in a survey conducted by the International Fragrance Association (IFRA, cited in Lake, 1999). The highest contribution to dermal exposure from one product category was 39%. It should be noted that this estimated dermal exposure makes no allowance for the evaporation of fragrance ingredients from the skin. Moreover, it is assumed that all dermally absorbed coumarin will be available for systemic circulation (Lake, 1999).

In a press release from 2007, BfR stressed that consumers could exceed the TDI of coumarin just by using cosmetics with high coumarin levels. The results were based on the levels of coumarin measured in random samples of cosmetics (mainly perfumes, but also skin gels, body lotions and oils). Regular use of leave-on-products with high levels of coumarin in children could exhaust up to 20 % of the TDI. Based on these results, BfR recommended that no coumarin should be used in care products for infants and toddlers as a precautionary measure (BfR, 2007).

PREVIOUS EXPOSURE ASSESSMENTS OF COUMARIN

The exposure to coumarin from food has previously been discussed in a review article by Lake (1999) and in scientific opinions from EFSA and BfR. A short description of the most important findings in these assessments is given below.

Lake (1999)
Lake calculated a Theoretical Added Maximum Daily Intake (TAMDI) of 4.1 mg coumarin/day (0.07 mg/kg bw/day for a person weighing 60 kg) based on intake data from food, beverages, caramel confectionery, chewing gum and alcoholic beverages. However, this theoretical estimated intake was described as unrealistically high.

A more reasonable assumption would be that over a long period of time less than 5% of solid food would be flavoured with cinnamon or other ingredients capable of imparting the 2 mg/kg maximum concentration of coumarin. Taking this assumption into account and considering an overall intake of 1.5 kg of solid food, the calculated maximum daily intake was reduced to about 1.2 mg/day (0.02 mg/kg bw/day for a 60 kg person).

European Food Safety Authority (EFSA) (2004) and (2008)
The EFSA AFC Panel calculated a TAMDI of 1.3-1.5 mg coumarin/day (equivalent to 0.02-0.025 mg coumarin/kg bw for an adult weighing 60 kg) in their opinion from 2004. EFSA's calculation was based on an assumed concomitant consumption of 324 g beverages in general, 133.4 g of food in general, 27 g of confectionary, 2 g of chewing gum and 20 g of alcoholic beverages, all containing coumarin at the current maximum permitted concentration.

EFSA also noted that exposure to coumarin from fragrances and other cosmetic products may be relatively high since the compound is readily absorbed by the dermal route (EFSA, 2004).

No exposure estimate was performed in the re-evaluated EFSA opinion on coumarin from 2008 (EFSA, 2008).

The German Federal Institute for Risk Assessment (BfR) (2006) and Abraham et al. (2010)
The German Federal Institute for Risk Assessment (BfR) has calculated the coumarin intake in children between 2 and 5 years. The evaluation showed that 140 of a total of 475 children in the food consumption study ate cinnamon or cinnamon-containing products at least one of the six days recorded (Banasiak *et al*., 2005, cited in BfR, 2006a). A consumption of 0.22 g/kg bw was reported for the 97.5 percentile among these consumers. Based on a coumarin level of 3000 mg/kg cinnamon, a worst case for oral coumarin exposure of 0.66 mg/kg bw was calculated (peak exposure due to consumption of rice pudding with cinnamon and sugar on individual days).

BfR also estimated the coumarin exposure in the same age group over a longer period (days to weeks). Based on certain assumptions, which take into consideration a higher intake of cinnamon-containing biscuits in the Christmas period, an exposure of 1.32 mg/kg bw/week (0.19 mg/kg bw/day) was estimated for the 2 to 5-year-olds.

When also taking into account the dermal exposure from cosmetics, BfR referred to a total daily exposure of 0.27 mg coumarin/kg bw. Their exposure assessment is based on worst case scenarios for both oral (0.19 mg) and dermal (0.08 mg) routes of exposure (BfR, 2006a).

As an approach to estimate the seasonally higher consumption of cinnamon-containing foods during Christmas time, BfR has also conducted a telephone survey where adults were interviewed about their average consumption of ten different cinnamon-containing foods (Abraham *et al.*, 2010). The mean coumarin intake was estimated to be 5.0 mg coumarin per week, while the median, 95th and 97.5th percentile intakes were 3.2, 15.9 and 21.6 mg coumarin per week, respectively. Six of the high consumers were found to have coumarin intakes above 35 mg per week, which would result in an estimated coumarin intake close to 0.1 mg/kg bw/day, when a bw of 60 kg is assumed (Abraham *et al.*, 2010).

SUMMARY OF EXPOSURE ASSESSMENTS

Total coumarin intake from cinnamon-containing foods based on data from the national food consumption surveys

The mean total intakes of coumarin from cinnamon-containing foods, presented in this opinion, range from 0.004 mg/kg bw/day in adults to 0.02 mg/kg bw/day in 4- and 9-year-old children. The coumarin intake among high consumers (95 percentile) was found to be highest in 4- and 9-year-old-children with estimated intakes of 0.06 and 0.05 mg/kg bw/day, respectively (Table 9a).

It should be noted that the intake calculations are influenced by many assumptions and should therefore be interpreted with caution. However, results from a recent research project on the total intake of cinnamon conducted at the University of Oslo indicate that the mean total coumarin intake from cinnamon-containing food (tea not included) for adults of 0.004 mg/kg bw/day calculated in this opinion is a reasonable estimate.

Total coumarin intake including cinnamon-based tea

Consumption of tea with a high content of cinnamon is an important source to the coumarin intake for persons preferring such tea, presumably adults. When cinnamon-based tea is included in the total intake calculations based on the data from the food consumption surveys, the mean total coumarin intake in adults increased to 0.05 mg/kg bw/day. Adults with a high coumarin intake (95 percentile) will have a total coumarin intake of 0.2 mg/kg bw/day due to the additional contribution from cinnamon-based tea (Table 9b). The assumed worst intake scenarios from cinnamon-based tea will lead to even higher coumarin intakes in adults, and also in other age groups (Table 12).

Coumarin intake from cinnamon powder sprinkled on oatmeal porridge and rice porridge

The intake from cinnamon powder sprinkled on oatmeal porridge or rice porridge is not included in the calculated total intake from the consumptions surveys. However, intake scenarios taking into account the amount and frequency of cinnamon powder consumed show that this is an important coumarin source, especially in small children consuming oatmeal porridge sprinkled with cinnamon powder.

The intake scenario for 1-year-old children that is based on a mean frequency of consumption of oatmeal porridge sprinkled with 1 ts of cinnamon powder results in a coumarin intake of 0.49 mg/kg bw/day, whereas the worst intake scenario (high frequency) with similar assumptions gives an intake of 1.63 mg/kg bw/day. Both 2-year-olds and 4-year-olds will also have high coumarin intakes based on a mean frequency of consumption, whereas all age groups were found to have high coumarin intakes with a high frequency of consumption of oatmeal porridge (Table 10).

The frequency of consumption of rice porridge is much less than for oatmeal porridge, but the contribution from cinnamon powder sprinkled on rice porridge will nevertheless be an important source to the intake of coumarin in all age groups (Table 11).

Assumed worst intake scenarios of specific cinnamon-containing foods

As described above, consumption of cinnamon powder sprinkled on porridge and cinnamon-based tea are the most important additional contributions to the total coumarin intake from food in the Norwegian population. Consumption of other cinnamon-containing food products, such as ginger bread, cinnamon buns and similar bakery products, thin pastry with cinnamon and cinnamon-containing cakes are of less importance to the coumarin intake. Assumed worst intake scenarios from all the different cinnamon-containing food categories included in the exposure assessment are shown in Tables 12-16.

Food supplements

High coumarin levels between 2180 and 3250 mg/kg capsule content were measured for three of the 14 analysed food supplements shown in Table 19. Taking into account the recommended daily dose, the coumarin intake from two of the cinnamon products was found to be 6 mg/day, equivalent to 0.1 mg/kg bw/day assuming a body weight of 60 kg. The overall results from all the analysed food supplements in the Norwegian survey showed coumarin intakes in the same order of magnitude as was found in a similar investigation in Germany in 2006.

Cosmetics

Exposure from cosmetic products is another important source that has to be taken into account when considering the total exposure to coumarin, as synthetic produced coumarin is added as fragrance to e.g. perfumes, shower gels, body lotions, deodorants and oils. A daily intake of 2.3 mg coumarin (0.04 mg/kg bw for an adult weighing 60 kg) has been estimated previously (Lake, 1999). This intake was considered realistic for a worst case scenario from cosmetics.

Comparison with previous exposure assessment of coumarin

The mean intake of coumarin in Norwegian adults (0.05 mg/kg bw/day when cinnamon-based tea is included) based on data from the national food consumption surveys is within the same range as previous exposure assessments of coumarin performed by Lake (0.07 mg/kg bw/day) and EFSA (0.02 – 0.025 mg/kg bw/day). Based on the data from the food consumption surveys, high consumers among Norwegian children aged 1-4-years have a lower intake of coumarin (0.02 – 0.07) from cinnamon-containing food than what has been reported in 2-5-year-old German children (0.19 mg/kg bw/day). However, Norwegian children who consume porridge sprinkled with cinnamon powder regularly could have coumarin intakes above what has been reported by BfR.

RISK CHARACTERISATION

The major toxicological findings were increased severity and incidence of nephropathy and a dose-dependent increase in absolute and relative liver weight in rats, and increased frequency of kidney adenomas in male rats in a 2-year study by NTP (see Table 3). A TDI of 0.07 mg/kg bw/day was established by the VKM Panel based on the lowest $BMDL_{05}$ (see Table 6) calculated for an increase in relative liver weight in rats using a safety factor of 100.

Total coumarin intake from cinnamon-containing foods based on data from the national food consumption surveys

The results from the intake calculations based on the data from the national food consumption surveys show that the mean total intake of coumarin from food is below TDI in all age groups, with 4- and 9-year-old children having the highest intakes of 0.02 mg/kg bw/day. This is independent of whether cinnamon-based tea is included in the calculations or not (Table 9a and 9b).

The coumarin intake among high consumers (95 percentile) who do not consume cinnamon-based tea is also below TDI for all age groups, although 4-year-old children are approaching TDI with an estimated coumarin intake of 0.06 mg/kg bw/day (Table 9a). The coumarin intake for tea drinking high-consumers reaches TDI for 4- and 9-year-olds children. Adults with a high consumption of cinnamon-containing foods will exceed the TDI of coumarin intake when tea consumption is included in the exposure estimations (Table 9b). It should be noted that the exceedance of TDI is only the case for children and adults that drink tea with a high content of cinnamon. Other brands of cinnamon-containing tea might contain considerably less coumarin than Yogi tea.

Total intake study of cinnamon in the Norwegian diet

A recent research project on the total intake of spices and herbs in the Norwegian diet has been conducted at the University of Oslo (M.H. Carlsen, L.F. Andersen and R. Blomhoff, manuscript in preparation). The calculated worst intake scenarios, based on a maximum amount and frequency of cinnamon consumption, result in coumarin intakes well above the TDI with both dietary methods used in the study (Table 18). Adults with a median amount and frequency of cinnamon consumption will have a coumarin intake in the range of 0.002 – 0.007 mg/kg bw/day, which is well below the TDI.

Coumarin intake from cinnamon powder sprinkled on oatmeal porridge and rice porridge

Cinnamon powder sprinkled on oatmeal porridge

For the mean frequency porridge consumption (Table 10), the coumarin intakes calculated in the different sprinkle scenarios (1/4 to 1 ts cinnamon powder) are all at or above the TDI of 0.07 mg/kg bw/day for coumarin for 1- and 2-year-old children. The sprinkle scenario with 1 ts of cinnamon powder at the mean frequency of porridge consumption is also above TDI for 4-year-old children.

For the high frequency porridge consumption (Table 10), the coumarin intakes calculated in the different sprinkle scenarios (1/4 to 1 ts cinnamon powder) are all above the TDI for coumarin for the 1- , 2- and 4-year-old children. Also for 9- and 13-year-olds and adults, the calculated coumarin intake exceeds TDI for the high frequency consumers when using 1-2 ts cinnamon powder to sprinkle the porridge.

With a mean frequency consumption of oatmeal porridge sprinkled with 1 ts of cinnamon powder, the 1- and 2-year-olds will exceed the TDI with 7-fold and 4-fold, respectively. Approximately 20-fold exceedance (1.63 mg/kg bw/day of coumarin) of TDI is reached when 1-year-old children have a high frequency consumption of oatmeal porridge sprinkled with 1 ts cinnamon powder. For 2-year-olds the same estimate leads to a 9-fold exceedance of TDI.

Cinnamon powder sprinkled on rice porridge

None of the age groups exceeded TDI for any of the sprinkle scenarios based on a mean frequency of rice porridge consumption (Table 11). For 1- and 2-year-olds with a high frequency of rice porridge consumption, the scenario using 1 ts of cinnamon powder to sprinkle the rice porridge leads to an exceedance of TDI, with an intake estimate of 0.11 and 0.9 mg coumarin/kg bw/day, respectively.

In summary, both a mean and high frequency of oatmeal porridge consumption in the age group 1- to 2-year-olds gives the largest contribution to the coumarin intake if the porridge is sprinkled with cinnamon powder.

The calculations show that adults would reach the TDI for coumarin of 0.07 mg/kg bw/day by consuming 1.4 g (approximately 1/2 ts) of cinnamon powder a day independent of which type of porridge is consumed. Children at the age of 4 and 1 years would reach TDI by consuming 0.4 g (approximately 1/6 ts) and 0.2 g (approximately 1/13 ts) cinnamon powder a day, respectively.

Assumed worst case scenarios of specific cinnamon-containing foods

A worst case intake scenario has been assumed for all relevant food items containing coumarin. For all food items, except cinnamon-based tea, the worst case intake estimates were at or below the TDI of coumarin in all age groups (Tables 12-16). The results from the assumed worst intake scenario of cinnamon-based tea show that all age groups will have an intake of coumarin above the TDI of 0.07 mg/kg bw/day, with a 6- and 3-fold exceedance of the TDI for adults and 13-year-olds respectively. It was anticipated that 1- and 2-year-old children do not drink tea.

Adults, who are assumed to have the highest consumption of tea in the population, were found to have an intake of 0.4 mg coumarin/kg bw/day by consuming four cups of cinnamon-based tea (Table 12). This implies that adults weighing 60 kg would reach the TDI of 0.07 mg coumarin/kg bw/day by consuming 3/4 cup of cinnamon-based tea (equivalent to 0.19 litre) daily.

An assumed worst case scenario for ginger bread which is consumed during the Christmas season did not result in an estimated intake that exceeded TDI for coumarin for any of the age groups (Table 13).

Coumarin intake from food supplements

Most of the food cinnamon supplements contained coumarin at levels which would not lead to exceedance of TDI at the recommended daily dose. For two of the cinnamon supplements containing coumarin, consumption of the recommended daily dose will by itself lead to a coumarin intake 1.4 fold above the TDI for adults (Table 19). It is not anticipated that children will consume supplements with cinnamon.

Coumarin intake from cosmetics

For adults, an estimated worst case coumarin intake from cosmetics of 0.04 mg/kg bw/day has been reported (Lake, 1999), which is below the TDI for coumarin. However, this is a considerable contribution to the total coumarin intake taking into account that this will be an addition to the estimated intake from food. It has been suggested that the peak blood concentrations of coumarin after dermal uptake might be lower that after oral exposure to the same dose. Accordingly, coumarin would be less hepatotoxic after dermal compared to oral exposure. Synthetic coumarin is used in cosmetic products used by children, such as shower gels, body lotions, deodorants and oils, but no exposure estimates for coumarin from cosmetics has been reported for children. Children have a higher surface to body weight ratio, and it is therefore anticipated that a worst case coumarin intake from cosmetics for children will be higher than the estimated value for adults. This is supported by the estimated worst case intake in children from cosmetics performed by BfR (BfR, 2006a; 2007).

Case reports of human exposure to coumarin

From a collection of human case reports from clinical treatment, a daily dose of 25 mg/person (corresponding to 0.4 mg/kg bw/day) as tablets for a few days was identified as the lowest dose documented to be capable of inducing hepatoxicity. Although these data is connected with some uncertainty since they are from a selective group of the population and the toxic effect level in animals is considerably higher than the TDI, the results indicate that 5-10 fold exceedance of TDI can result in toxic effects in humans. The case reports also indicate that a coumarin intake several fold higher than TDI for a few weeks might represent a risk for consumers.

SUMMARY OF RISK CHARACTERISATION

The highest contribution to the intake of coumarin, both for children and adults, came from oatmeal porridge sprinkled with cinnamon powder and from cinnamon-based tea with high levels of coumarin. Small children (1- and 2-year olds) who have a mean or high frequency of oatmeal porridge consumption will be at risk for exceeding TDI up to about 20-fold, even if they use moderate amounts of cinnamon powder on the porridge regularly. For high consumers of oatmeal porridge among small children who sprinkle with high amounts of cinnamon powder, the estimated coumarin intake can exceed the levels reported to give liver toxicity in human case reports.

Drinking of cinnamon-based tea can also result in a total intake of coumarin that exceeds TDI both for children and adults. It should be noted that the exceedance of TDI is only the case for children and adults that drink tea with a high content of cinnamon. Some brands of cinnamon-containing tea might contain lower levels of coumarin than Yogi tea.

In addition, coumarin in cosmetics might contribute considerably to the total coumarin intake. Coumarin exposure from cosmetics is only reported for adults. It is anticipated that children will have a higher exposure to coumarin from cosmetics than adults due to a higher surface to body weight ratio.

The VKM Panel concludes that based on the available data, the possibility of an adverse health effect by exceeding the TDI 3-fold for 1-2 times per week for several years cannot be

assessed. Generally, a minor or an occasional exceedance of TDI is not considered to increase the risk of adverse health effects.

The coumarin intake could exceed the TDI by 7-20 fold in some instances. Liver toxicity may occur shortly after the start of coumarin exposure. Such large daily exceedances of TDI, even for a limited time period of 1-2 weeks, cause concern of adverse health effects.

UNCERTAINTY AND VARIABILITY

HAZARD IDENTIFICATION AND CHARACTERISATION

The hazard identification and characterisation are based on extensive toxicological data from several animal species, and human case reports. The VKM Panel noted considerable species differences in the biotransformation of coumarin, with rats as the most sensitive species. Dose reponses were modelled, and BMDs and BMDLs were estimated from a long-term toxicity study in rats, and used as point of departures in the derivation of the TDI.

DIETARY EXPOSURE ASSESSMENT

The risk assessment of coumarin presented in this opinion is affected by scientific uncertainties in the dietary exposure assessment, as well as uncertainty in varying concentrations of coumarin in different food items. The Guidance of the EFSA Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006) was considered when describing some of the inherent uncertainties and variability in this opinion from VKM.

According to the guidance provided by the EFSA opinion (EFSA, 2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model and model input.

Assessment objectives
The mandate of the risk assessment was clearly specified in the terms of reference, and the VKM Panel 4 has assessed the occurrence data for coumarin in different food items and supplements provided by the Norwegian Food Safety Authority. The different approaches used in the exposure assessment were considered sufficient to perform the risk assessment and prepare the opinion.

Exposure scenario
The exposure to coumarin through the consumption of cinnamon-containing foods has been the main focus of this opinion. Additional oral exposure through cinnamon-containing food supplements has also been discussed. The VKM Panel 4 is of the opinion that the sources of coumarin exposure in the Norwegian diet are described as completely as possible, and it is not expected that important sources are missing. The information on which dietary sources is relevant is based on a selection of monitoring programmes and analyses of the coumarin levels in different cinnamon-containing foods, performed by many EU member states.

It should however be noted that the dermal route of exposure through the use of cosmetics could be an important source when estimating the total exposure to coumarin. As there are no

available occurrence data for coumarin from cosmetics sold on the Norwegian market, the VKM Panel 4 has referred to relevant literature for dermal exposure.

The intake calculations for coumarin from the different food and drink categories presented in this opinion bring some uncertainties, due to e.g. a limited number of analysed products and a lack of consumption data for the specific food items that contain cinnamon (se more detailed information in the section "Model input").

Exposure model

A generally accepted model for estimation of food exposure has been used in the exposure assessment. The model considers consumption data for relevant food and drink categories in the national representative dietary surveys and also the analytical data of coumarin levels in different food items provided by the Norwegian Food Safety Authority. In such dietary surveys, information about food consumption among individuals is collected using a dietary assessment method. It should be noted that the food consumption surveys only estimate the general diet in the population. Data on specific food products and brands is usually not included in these surveys. Due to the lack of consumption data for some specific food items that contain cinnamon evaluated in this opinion, the VKM Panel 4 has also presented some assumed "worst intake scenarios" from various cinnamon-containing food products, thought to be important sources to cinnamon-exposure in the Norwegian diet.

Consumers only

The coumarin intake calculated from the data in the food consumption surveys is based on consumers only of cinnamon-containing products or similar products as described in this opinion. If all the participants in the food consumption surveys were included in the calculations, the intake could be underestimated among those who actually consume the foods.

Model input

Coumarin levels in cinnamon

There are several species of cinnamon, and the content of coumarin has been found to vary considerably both between various species and also in different parts of the cinnamon tree (bark, leaves, roots). It is especially important to differentiate between Ceylon cinnamon and Cassia cinnamon as the chemical composition of the two types is different (BfR, 2006a). The coumarin levels in cinnamon powder (Cassia cinnamon) have been shown to vary from 1500-8000 mg/kg. Cinnamon powders analysed both in the Norwegian surveys and previously by BfR in Germany had coumarin levels in the range 2300 - 3330 mg/kg. This natural variability in coumarin levels brings uncertainty to the exposure assessment. The calculations in this opinion from VKM are based on a mean value of 3000 mg coumarin/kg cinnamon powder (Abraham *et al*., 2010).

Sampling uncertainty in the analytical data

The average and maximum coumarin levels in the cinnamon-containing food items used in the different exposure scenarios are based on measures of only a small number of samples (single analyses of 1-6 different brands). It should therefore be noted that samples showing high coumarin levels (e.g. for cinnamon-containing cakes, see Table 7 and Appendix I) would result in high average values compared to the median. However, the average coumarin levels presented in Table 7 are considered as an appropriate basis for the calculations of the total coumarin intake from the national food consumption surveys.

Consumption data

It is important to interpret the results of the exposure assessment in this opinion with great caution as there are many assumptions and uncertainties in the consumption data. A description of the most important assumptions in the intake calculations and how they could bring uncertainty to the results is described below.

The coumarin levels from the analysed foods have been extrapolated to similar food categories in the national food consumption surveys, as consumption data are lacking for the specific food item in question. This assumption has been necessary to do for the food categories ginger bread, cinnamon buns and cinnamon-containing cakes, where the coumarin intake has been based on the reported consumption of ginger nuts and biscuits, similar bakery products and similar cakes, respectively.

Some of these assumptions could lead to an overestimation of the intake, while others could result in an underestimation. For cinnamon buns, the assumption is considered realistic for individuals who prefer such products when they consume bakery products, but would in general lead to an overestimation of the coumarin intake.

Another example is that none of the Norwegian food consumption surveys include data on the consumption of cinnamon-based tea as such. The coumarin intake from cinnamon-based tea is therefore based on the assumption that available data on all tea consumption in the food consumption surveys is replaced by similar amounts of cinnamon-based tea. Such an assumption is not considered very likely for the whole population, but could be a realistic approach for those persons who actually prefer cinnamon-based tea.

The intake calculations for coumarin from cinnamon powder sprinkled on oatmeal porridge and rice porridge should also be interpreted with caution because of uncertainties in the estimates. The frequency of consumption of porridge for 4-, 9- and 13-year-old children is only assumptions, since it was not possible to calculate due to the design of the food consumption survey. It should further be noted that there is probably a huge variation in the amount and frequency of consumption of cinnamon (number of teaspoons of cinnamon powder used) depending on which taste is preferred by different individuals. The assumption that cinnamon powder is used on all oatmeal porridge which is consumed as presented in the intake calculations is considered an overestimate for the general population, but not for consumers who prefer to use cinnamon powder on their porridge. This is in accordance with calculations from a total intake study of cinnamon among Norwegian adults conducted at the University of Oslo, which show that consumption of rice porridge correlates with the consumption of cinnamon, whereas no such correlation was found between the consumption of oatmeal porridge and consumption of cinnamon (M.H. Carlsen, L.F. Andersen and R. Blomhoff, manuscript in preparation).

Body weight

The dietary exposure to coumarin in this opinion is estimated relative to body weight (mg/kg bw/day) for all age groups. The individual consumption data taken from the food consumption surveys should ideally be paired with data on body weights for the same individuals. However, this was not possible for all the intake calculations that were carried out in this opinion. It was therefore decided to use the mean body weights for the different age

groups in all calculations. When mean body weights are combined with individual data on consumption, the assessment may overestimate the degree of individual variation in dietary exposure for some foods, if their consumption is correlated with body weight.

For adults, a default body weight of 60 kg was used. This could lead to an overestimation of the intake when it is combined with individual data on consumption, since the mean body weight for adults are higher than 60 kg for both Norwegian men and women.

SUMMARY OF UNCERTAINTIES

An evaluation of the overall effect of identified uncertainties is presented in Table 20, 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.

Table 20. *Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of coumarin.*

Source of uncertainty	Direction and magnitude*
Hazard characterisation	+/-
Variation in coumarin levels in cinnamon	+/-
Measurement uncertainty due to a limited number of samples	+/-
Extrapolation of coumarin levels from the analysed foods to similar food categories in the dietary surveys	+/-
Amount and frequency of consumption of cinnamon powder used on oatmeal porridge by the general population	+
Combination of mean body weights with individual data on consumption from the food consumption surveys	+
Default body weight of 60 kg	+
Exposure from the dermal route	-

* + = Uncertainty with potential to cause overestimation of exposure/risk; - = Uncertainty with potential to cause underestimation of exposure/risk.

The VKM Panel concluded, after an overall evaluation of uncertainties, that by using the upper end of the high exposure estimate and the lower end of the $BMDL_{05}$ estimates, the risk assessment of coumarin is likely to be conservative, i.e. more likely to overestimate than to underestimate the risk.

CONCLUSIONS

- The VKM Panel has evaluated the available data on toxicity of coumarin. The most significant hazards of coumarin appears to be liver toxicity, which is well documented, and demonstrated in mice, rats, dogs, baboons and humans, and kidney adenomas in male rats. A small subgroup of the human population appears for unknown reasons to be more susceptible to medical treatment with coumarin. The lowest reported dose of coumarin associated with liver toxicity in humans is around 0.4 mg/kg bw/day. It should be noted that the liver toxicity of coumarin in humans usually is reversible. Since there were no dose-response data for humans, animal data were used in the hazard characterisation. The VKM Panel used the $BMDL_{05}$ of 7 mg/kg bw/day (converted from 10 mg/kg bw, 5 times per week) for relative increase in liver weight in a long-term study in rats to establish a TDI of 0.07 mg/kg bw/day, using an uncertainty factor of 100 to account for inter- and intraspecies variation.

- The total estimated intake of coumarin for mean and high consumers of cinnamon-containing foods were below the TDI for all age groups when consumption of cinnamon-based tea and porridge with cinnamon was excluded.

- Children and adults who regularly consume oatmeal porridge sprinkled with cinnamon may exceed the TDI by several folds depending on the frequency of consumption and the amount of cinnamon used.

- Small children (1- and 2-years old) who have a mean or high consumption of oatmeal porridge may exceed the TDI even if they use moderate amounts of cinnamon powder on the porridge. In a worst case scenario with high consumption of porridge and use of high amounts of cinnamon powder, the estimated coumarin intake could exceed the TDI by about 20-fold. This intake is similar to dose levels of coumarin used in medical treatment of adults and where cases of liver toxicity have been reported.

- Drinking of cinnamon-based tea, which may have a high content of coumarin, can also result in a total intake of coumarin that exceeds the TDI both for children and adults.

- Other relevant sources of coumarin are cosmetics and food supplements with cinnamon. The recommended dose of two cinnamon supplements sold on the Norwegian market can lead to an exceedance of TDI in adults. It is not anticipated that children will consume supplements with cinnamon. Cosmetic products (shower gels, body lotions, deodorants and oils) are important sources of coumarin exposure both for children and adults, but quantification of the coumarin exposure from cosmetics was not possible due to lack of data.

- The VKM Panel concludes that based on the available data, the possibility of an adverse health effect by exceeding the TDI 3-fold for 1-2 times per week for several years cannot be assessed. Generally, a minor or an occasional exceedance of TDI is not considered to increase the risk of adverse health effects.

- The coumarin intake could exceed the TDI by 7-20 fold in some instances. Liver toxicity may occur shortly after the start of coumarin exposure. Such large daily exceedances of TDI, even for a limited time period of 1-2 weeks, cause concern of adverse health effects.

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APPENDICES

APPENDIX I

Table A1. *Coumarin levels in cinnamon and cinnamon-containing products sold on the Norwegian market, survey conducted by the Norwegian Food Safety Authority in 2008 (brand names in Norwegian).*

No.	Product	Manufacturer	Best-before date	Coumarin level (mg/kg)
1	Figurbakte pepperkaker	Sætre	12.10.2009	17
2	Skillingsboller	Møllerens	13.08.2009	12.7
3	Solskinnsboller	Møllerens	16.08.2009	13.8
4	Pepperkakehus	Annas	21.10.10 og 31.01.10	10.5
5	Pepperkaker i boks	Bjørken	12.10.2009	22.9
6	Vestlandslefse med sukker og kanel	Rieber & Søn	Okt. 2009, L 8295 og L8290	8.6
7	Vestlandslefse med eple og kanel	Rieber & Søn	Mai 2009, L 8142	2.6
8	Tomtegløgg godt krydret	Sara Lee	09.09.2012	<1[a]
9	Tomtegløgg	Sara Lee	12.08.12 og 14.08.12	<1[a]
10	Pepperkakehjerte i boks	Berthas	25.09.2009	15.2
11	Fibra krydderkake	Møllerens	07.04.2009	40.2
12	Twinings te med eple, kanel, rosin	Twinings	28.09.11 og 28.08.11	<1[a]
13	Kanelboller	Findus	12.09 L08270	5.9
14	Pågen gifler kanel	Pågen	16.12.08, bakt 21.10.08	22.6
15	Pågen kanelboller	Pågen	19.12.2009	43.9
16	Coop ferske kanelboller	Goman bakeriet		9.5
17	Smurte lefser, glutenfri	Stjørdals glutenfri hjemmebakeri	07.07.09 og 16.07.09	3.7
18	Tykklefse	Lierne bakeri	13.01.09 og 03.02.09	3.4
19	Smurte lefser	Berthas	09.02.2009	20.1
20	Krydderikage	Dan Cake		4.7
21	Liklenning med kanel	Lierne	09.04.2009	2.1
22	Hel kanel	Santa Maria	23.10.2013	49.9
23	Malt kanel	Santa Maria	28.10.2011	2350
24	Malt kanel, økologisk	Santa Maria	18.09.2011	3330

[a]Below the indicated limit of quantification (LOQ).

Table A2. *Coumarin levels in cinnamon capsules and other cinnamon-containing products sold on the Norwegian market, survey conducted by the Norwegian Food Safety Authority in 2009.*

No.	Product/Manufacturer	Batch number	Best-before date	Coumarin level (mg/kg)
1	Lamberts Cinnamon	102637	05/2010	371
2	Lamberts Multi-Guard Control	104794	04/2011	155
3	NutriFood Kanel	8042101 9020502 8091601		607
4	Nature's Way Cinnamon Standardized	541575 565793	05-2010 03-2011	2320
5	Salus Momordica	A95001 A95004	02/2010 02/2010	4.75
6	Gevita Kanel	010709	07-2011	<1
7	Swanson Cinnamon Extract	171260	05/11	613
8	DFI Kanel+	9939-2	08-2011	235
9	Solaray Cinnamon Bark	130707	12/12	924
10	Swanson Cinnamon Gymnema Mulberry Complex	170582	05/11	3250
11	Swanson Full Spectrum Cinnamon	171636	06/11	2180
12	Swanson Cinnulin PF	170186	04/11	804
13	Bio Insu Complex Kanelbarkekstrakt		12/5 2012 12/5 2012	146
14	New Nordic DIDA Kanelolje	04407 06307 14108	04-2010 04-2010 10-2011	<10
15	Suma Organic Cinnamon Bark		20.06.2011 27.12.2010	4070
16	Natur-Drogeriet knust kanelbark	8309010	17.11.2011	37.9
17	Yogi Tea Classic (løs vekt, 55% cinnamon)		13.11.2011	22.7
18	Yogi Tea Classic (teposer, 61% cinnamon)		13.05.2012 24.01.2012 24.04.2012	9.65

APPENDIX II

COUMARIN INTAKE FROM CINNAMON-BASED TEA

Table A3 shows the mean and high (95 percentile) intake of coumarin from cinnamon-based tea for different age groups in the Norwegian population (consumers only). The calculated intake is based on the mean level of 16.2 mg coumarin/kg found in Yogi tea (see Table 7). The coumarin levels are measured in prepared tea (ready to drink).

Table A3. *Coumarin intake from cinnamon-based tea for different age groups (consumers only).*

		Consumption (g/day)		Coumarin intake (mg/kg bw/day)[a]	
Age groups	**N (%)[b]**	**Mean**	**95 percentile**	**Mean**	**95 percentile**
1-year-olds[c]	-	-	-	-	-
2-year-olds[c]	-	-	-	-	-
4-year-olds	28 (7%)	62	100	0.06	**0.09**
9-year-olds	84 (10%)	84	200	0.04	**0.10**
13-year-olds	165 (16%)	91	240	0.03	**0.08**
Adults	1868 (70%)	215	700	0.06	**0.19**

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).
[c]Consumption of cinnamon-based tea not considered likely.

COUMARIN INTAKE FROM GINGER BREAD

Table A4 shows the mean and high (95 percentile) intake of coumarin from ginger bread for different age groups in the Norwegian population (consumers only). The calculated intake is based on the mean level of 16.4 mg coumarin/kg found in ginger bread (see Table 7).

Table A4. *Coumarin intake from ginger bread for different age groups (consumers only).*

		Consumption (g/day)		Coumarin intake (mg/kg bw/day)[a]	
Age groups	**N (%)[b]**	**Mean**	**95 percentile**	**Mean**	**95 percentile**
1-year-olds	704 (43%)	2.4	9	0.004[c]	0.02[c]
2-year-olds	1108 (66%)	3.1	10	0.004[d]	0.01[d]
4-year-olds	162 (41%)	5.7	15	0.005	0.01
9-year-olds	181 (22%)	8.7	29	0.004	0.01
13-year-olds	98 (10%)	7.3	23	0.002	0.007
Adults	1121 (42%)	5.4	22	0.002	0.006

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).
[c]Consumers included in the coumarin calculations (N=651).
[d]Consumers included in the coumarin calculations (N=1084).

COUMARIN INTAKE FROM CINNAMON BUNS AND SIMILAR BAKERY PRODUCTS

Table A5 shows the mean and high (95 percentile) intake of coumarin from cinnamon buns and similar bakery products for different age groups in the Norwegian population (consumers only). The calculated intake is based on the mean level of 18 mg coumarin/kg found in cinnamon buns and similar bakery products (see Table 7).

Table A5. *Coumarin intake from cinnamon buns and similar bakery products for different age groups (consumers only).*

		Consumption (g/day)		Coumarin intake (mg/kg bw/day)[a]	
Age groups	**N (%)[b]**	**Mean**	**95 percentile**	**Mean**	**95 percentile**
1-year-olds	874 (53%)	2.4	7	0.004	0.01
2-year-olds	1245 (74%)	4.5	15	0.006	0.02
4-year-olds	134 (34%)	23	51	0.02	0.05
9-year-olds	319 (39%)	31	88	0.02	0.05
13-year-olds	320 (32%)	41	100	0.02	0.04
Adults	1968 (74%)	12	34	0.004	0.01

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).

COUMARIN INTAKE FROM THIN PASTRY WITH CINNAMON

Table A6 shows the mean and high (95 percentile) intake of coumarin from thin pastry with cinnamon for different age groups in the Norwegian population (consumers only). The calculated intake is based on the mean level of 6.8 mg coumarin/kg found in thin pastry with cinnamon (see Table 7).

Table A6. *Coumarin intake from thin pastry with cinnamon for different age groups (consumers only).*

		Consumption (g/day)		Coumarin intake (mg/kg bw/day)[a]	
Age groups	**N (%)[b]**	**Mean**	**95 percentile**	**Mean**	**95 percentile**
1-year-olds[c]	-	-	-	-	-
2-year-olds[c]	-	-	-	-	-
4-year-olds	10 (3%)	15	30	0.006	0.01
9-year-olds	19 (2%)	13	18	0.003	0.004
13-year-olds	12 (1%)	24	49	0.003	0.007
Adults[c]	-	-	-	-	-

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).
[c]No consumption data of thin pastry with cinnamon available in the dietary surveys.

COUMARIN INTAKE FROM CINNAMON-CONTAINING CAKES

Table A7 shows the mean and high (95 percentile) intake of coumarin from cinnamon-containing cakes for different age groups in the Norwegian population (consumers only). The calculated intake is based on the mean level of 22.5 mg coumarin/kg found in cinnamon-containing cakes (see Table 7).

Table A7. *Coumarin intake from cinnamon-containing cakes for different age groups (consumers only).*

		Consumption (g/day)		Coumarin intake (mg/kg bw/day)[a]	
Age groups	**N (%)[b]**	**Mean**	**95 percentile**	**Mean**	**95 percentile**
1-year-olds[c]	-	-	-	-	-
2-year-olds[c]	-	-	-	-	-
4-year-olds	59 (15%)	16	38	0.02	0.05
9-year-olds	114 (14%)	18	38	0.01	0.03
13-year-olds	100 (10%)	23	75	0.01	0.03
Adults[c]	772 (29%)	4	11	0.002	0.004

[a]Estimated based on the mean body weight for the different age groups. For adults, a default body weight of 60 kg was used.
[b]N (%) = number of persons with a consumption of the relevant food category (percentage of all consumers in the consumption survey).
[c]No consumption data of cinnamon-containing cakes available in the dietary surveys.

APPENDIX III

Table A8 shows the mean and high (95 percentile) frequencies of consumption of oatmeal porridge and rice porridge for 1- and 2-year-old children and adults. The frequencies of consumption for the participants in the dietary surveys who had a consumption of both oatmeal porridge and rice porridge are also included for these age groups.

Table A8. *Frequency of consumption of oatmeal porridge and rice porridge among 1-year-olds, 2-year-olds and adults (consumers only).*

	Frequency of consumption				
	Oatmeal porridge			Rice porridge	
Age groups (n=consumers)	**Mean (times/ week)**	**95-percentile (times/ week)**	**Age groups (n=consumers)**	**Mean (times/ week)**	**95-percentile (times/ week)**
1-year-olds (n=254)	4.2	14	1-year-olds (n=401)	0.5	1.0
1-year-olds[a] (n=98)	3.6	14	1-year-olds[a] (n=98)	0.5	1.0
2-year-olds (n=565)	3.1	7	2-year-olds (n=954)	0.6	1.0
2-year-olds[a] (n=348)	2.9	7	2-year-olds[a] (n=348)	0.6	1.0
Adults (n=700)	1.1	4.5	Adults (n=1879)	0.4	0.9
Adults (n=593)[a]	1.0	4.5	Adults (n=593)[a]	0.5	1.0

[a]Consumers of both oatmeal porridge and rice porridge.

It has not been possible to provide exact data on the frequency of consumption of oatmeal porridge and rice porridge for 4-, 9- and 13-year-olds as the food consumption survey for these age groups (Ungkost 2000) is based on a 4-day food consumption registration. The intake of coumarin from oatmeal porridge and rice porridge in these age groups has therefore been calculated on the basis of the assumed frequencies shown in Table A9.

Table A9. *Assumed frequency of consumption of oatmeal porridge and rice porridge among 4-year-olds, 9-year-olds and 13-year-olds (consumers only).*

	Frequency of consumption			
	Oatmeal porridge		Rice porridge	
Age groups (n=consumers)	**Mean (times/week)**	**High (times/week)**	**Mean (times/month)**	**High (times/month)**
4-year-olds	2	5	2	4
9-year-olds	1	3	2	3
13-year-olds	0.5	3	2	3