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REVIEW

# Toxicology and risk assessment of coumarin: Focus on human data

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Coumarin is a secondary phytochemical with hepatotoxic and carcinogenic properties. For the carcinogenic effect, a genotoxic mechanism was considered possible, but was discounted by the European Food Safety Authority in 2004 based on new evidence. This allowed the derivation of a tolerable daily intake (TDI) for the first time, and a value of 0.1 mg/kg body weight was arrived at based on animal hepatotoxicity data. However, clinical data on hepatotoxicity from patients treated with coumarin as medicinal drug is also available. This data revealed a subgroup of the human population being more susceptible for the hepatotoxic effect than the animal species investigated. The cause of the high susceptibility is currently unknown; possible mechanisms are discussed. Using the human data, a TDI of 0.1 mg/kg body weight was derived, confirming that of the European Food Safety Authority. Nutritional exposure may be considerably, and is mainly due to use of cassia cinnamon, which is a popular spice especially, used for cookies and sweet dishes. To estimate exposure to coumarin during the Christmas season in Germany, a telephone survey was performed with more than 1000 randomly selected persons. Heavy consumers of cassia cinnamon may reach a daily coumarin intake corresponding to the TDI.

**Keywords:**
Coumarin / Exposure / Humans / Risk assessment / Toxicology

## 1 Introduction

Coumarin (1,2-benzopyrone, CAS No. 91-64-5) consists of an aromatic ring fused to a condensed lactone ring (Fig. 1). It is a naturally occurring constituent of many plants with a pleasant spicy odor of fresh hay, woodruff or vanilla. Along with safrole and estragole, it belongs to the group of ingredients in spices and herbs that are listed by the Council of Europe as ''active principles''. In foods they may have a strong flavor but they are also toxicologically relevant.

Coumarin has a long and interesting history of use and regulation. In 1822, the substance was isolated and purified for the first time from tonka beans (seed of *Dipteryx odorata*, also called *Coumarouna odorata*). After chemical synthesis in 1868, coumarin was marketed and used as a food flavoring for a long time [1]. In the middle of the last century, coumarin was discovered to cause hepatic damage in laboratory animals [2], and the addition of synthetic coumarin to foods was banned, first in the USA in 1954. Furthermore, the formation of tumors was observed in long-term animal experiments [3], and for a long time, a genotoxic mechanism of action could not be ruled out. According to the as low as reasonably achievable principle, in 1988 the European Union set a strong coumarin limit of 2 mg/kg for food in general resulting from the use of natural spices and herbs, with exceptions for special foods (chewing gum, caramel confectionery, and alcoholic beverages). As for other ''active principles'' regulated in the council directive 88/388/

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**Abbreviations: BfArM,** Federal Institute for Drugs and Medical Devices; **BfR,** Bundesinstitut für Risikobewertung; **EFSA,** European Food Safety Authority; **o-HPA,** *o*-hydroxyphenylacetaldehyde; **o-HPAA,** *o*-hydroxyphenylacetic acid; **NOAEL,** no observed adverse effect level; **TAMDI,** theoretical added maximum daily intake; **TDI,** tolerable daily intake

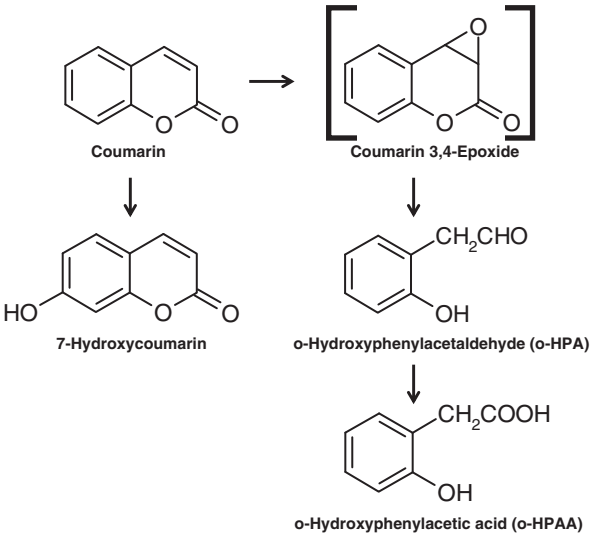


**Figure 1.** Major metabolic pathways (phase I) of coumarin.

EEC [4], the value of 2 mg/kg represented the limit of detection at that time. During the following years, however, compliance with this limit was evidently not monitored closely by food regulatory authorities in Europe. In 2005, the Chemisches und Veterinäruntersuchungsamt in Münster, Germany, discovered a coumarin content of 22 mg/kg by chance in a sample of cinnamon star cookies, (''Zimtsterne'', a typical German Christmas cookie) www.bfr.bund.de/cm/245/high_daily_intakes_of_cinnamon_health_risk_cannot_be_ruled_out.pdf [5]. This prompted a great increase in coumarin measurements in food from the German market [6], and a political debate on product recalls and the level of compliance with regulatory limits by the food industry in Europe [7]. As a result of these investigations and discussions, new coumarin limits for cinnamon-containing foods were laid down in the European Regulation EC 1334/2008, which replaced the earlier Directive 88/388/EEC.

These changes in the regulation of coumarin also reflect changes in the scientific understanding of coumarin toxicology. On the European level, comprehensive opinions of the former Scientific Committee on Food of the European Commission are available from 1994 and 1999. The Committee could not rule out a genotoxic mode of action for tumor formation and recommended a maximum level of coumarin of 0.5 mg/kg in foods. These opinions were revised by the Panel of European Food Safety Authority (EFSA) (the successor of the Scientific Committee on Food) in 2004 [4] on the basis of new evidence for a non-genotoxic mode of action. This made it possible to derive a tolerable daily intake (TDI) for the first time.

In the past, reports and opinions dealing with the question of risk assessment for coumarin focused primarily on animal data and its extrapolation to humans [3, 4, 8]. However, considerable human data, indicating hepatotoxicity, is available from use of coumarin as a medicinal drug. In this review, we take a closer look at this clinical data, which in our view has not been sufficiently evaluated before, and use this information to derive a TDI. We also survey the possible causes of coumarin hepatotoxicity in a subgroup of the human population. Coumarin exposure is discussed in the light of data on coumarin content in cinnamon-containing foods from Germany, and on new consumption data from a telephone survey during the Christmas season. This information allows us to make a risk assessment for coumarin.

## 2 Hazard of coumarin

### 2.1 Data from laboratory animals

Extensive data is available on the toxicity of coumarin in laboratory animals, and these have been evaluated in various overview articles and expert opinions of scientific bodies. Of the effects observed *in vivo*, the carcinogenic and hepatotoxic properties are of major importance. Hepatotoxicity was observed not only in rodents, but also in many other mammal species. Tumor formation was observed in long-term experiments with rodents: adenomas and carcinomas of the liver and bile ducts and adenomas of the kidney in rats, as well as adenomas and carcinomas of the lung and liver adenomas in mice. Carcinomas were found only at doses higher than 100 mg/kg body weight *per* day. Since the findings on the carcinogenic properties of coumarin in the 1960s, there had been discussions about their importance for humans and the underlying mechanism of action [3, 4]. After evaluating of new data on DNA adduct formation in 2004 [9–11], EFSA concluded that *in vivo*, coumarin does not bind in a covalent manner to the DNA of target organs and therefore that its carcinogenic effect does not have a genotoxic mechanism. Instead, coumarin induces tumors by a mechanism, which is preceded by toxicity in the same target organ, and this allows a threshold-based approach and the establishment of no observed adverse effect level (NOAEL) [4]. After evaluation of the available oral animal studies for sub-acute and chronic toxicity, hepatotoxicity in Beagle dogs [12] was identified as the most sensitive effect. In this study, hepatotoxic effects were evident in the animals given 25 mg/kg body weight daily, but not in the animals given 10 mg/kg body weight daily (autopsies between day 297 and day 350). These results were used to establish a NOAEL of 10 mg/kg body weight daily; by using a safety factor of 100 (10 for interspecies variation and 10 for interhuman variability), a TDI of 0.1 mg/kg body weight daily was derived [4].

As to the mechanism of hepatotoxicity, many *in vivo* and *in vitro* studies have been performed in laboratory animals to elucidate the metabolism of coumarin [3, 4]. Briefly, the two most important pathways of coumarin metabolism are 7-hydroxylation leading to detoxification, which is predominant in primates [13], and metabolism of the lactone ring to form a coumarin 3,4-epoxide intermediate (Fig. 1). This

 

can be conjugated with glutathione or may spontaneously degrade with the loss of carbon dioxide to form *o*-hydroxyphenylacetaldehyde (*o*-HPA). The latter compound was found to be a hepatotoxic metabolite and is detoxified by oxidation to *o*-hydroxyphenylacetic acid (*o*-HPAA). Much less *o*-HPAA is formed in rats than mice, explaining the higher susceptibility of rats to coumarin-induced hepatotoxicity. Therefore, differences in detoxification of *o*-HPA are assumed to be a determining factor for species differences in sensitivity to coumarin hepatotoxicity [14].

### 2.2 Human experience from coumarin use as medicinal drug

Coumarin was approved from the 1970s onwards in various countries as a medicinal product to treat edemas caused by venous (chronic venous insufficiency) and lymphatic (lymphatic edemas) drainage disorders, possibly through stimulation of proteolysis by tissue macrophages. In addition, direct anti-tumor activity was reported, and the substance was used to treat renal cell carcinoma and other tumors, with doses of up to 7000 mg daily [15]. Some of the patients developed severe hepatotoxicity (toxic hepatitis, liver failure in a few cases) from a few weeks to 6 months after commencement of treatment [16–18]. These observations led to these products being withdrawn from the market in several countries in the 1990s (Australia, Belgium, France and Canada) [18].

Varying frequencies of the hepatotoxic response were reported, with dependence on the method used to detect hepatotoxicity (clinical observation only or blood sampling to detect elevated liver enzyme levels), as might be expected. In one report, 3 of 48 patients with metastatic prostatic carcinoma (6.3%) treated with 3000 mg coumarin daily responded with elevated liver enzymes [19]. Cox *et al.* observed a study group of 2173 patients with cancer or chronic infections; they were treated with 25–2000 mg coumarin daily (the majority received 100 mg daily for 1 month, followed by 50 mg daily for 2 years; blood samples were taken every 3 months). Seventeen patients developed elevated liver enzymes levels of sufficient magnitude (*i.e.* alanine aminotransferase levels between 115 and 960 U/L, at least double the normal maximum level of 35–50 U/L). Four of them were diagnosed as probably having other causes than coumarin treatment. Of the remaining 13 patients (0.60%), elevated liver enzymes levels returned to normal while still on coumarin in five, and only in the remaining eight (0.37%) was the hepatotoxicity attributed to coumarin treatment. Several of the patients were re-exposed to coumarin showing the same hepatotoxic response, but with a faster onset (Section 2.4) [16].

A more reliable estimate of the percentage of patients sensitive to the hepatotoxic effect of coumarin can be expected from systematic placebo-controlled studies with recurrent blood sampling. Loprinzi *et al.* studied 140 women with chronic lymphedema of the ipsilateral arm after treatment for breast cancer [20]. They received 200 mg coumarin or placebo twice daily for 6 months and then the other medication for the following 6 months (cross-over design). Blood samples were taken for the first time after 3 to 4 months and after 1 month during the first and second treatment period, respectively. The incidence of hepatotoxic effects was substantially higher with coumarin than with placebo. In none of the women did serum aminotransferase levels reach 2.5 times the upper limit of normal during the placebo period, whereas in nine women (6%) the levels became high during treatment with coumarin ($p = 0.006$). The most prominent instance of hepatotoxicity occurred in a woman who developed jaundice with a serum bilirubin concentration of 19.3 mg *per* deciliter while she was receiving coumarin. In these nine women, the enzyme levels returned to normal after coumarin treatment was stopped. The authors were unable to identify any predisposing factors, excluding, among others, therapy with tamoxifen and high body weight.

In a clinical study from Germany [21–23], 231 patients with chronic venous insufficiency were randomly assigned verum (90 mg coumarin and 540 mg troxerutin per day, $n = 114$) or placebo ($n = 117$) for 16 weeks. Blood liver enzymes were monitored at baseline and at five time points during the treatment. Four weeks after the beginning of the therapy, nine out of the 114 patients treated with verum (7.9%) manifested elevated transaminase levels above 2.5-fold the upper limit of the normal range. They were analyzed with respect to causality: three were assessed as ''unrelated'', two as ''unlikely'', three as ''possible'' and one as ''probable''. Elevated liver enzymes were also observed in the placebo group (number not reported). The authors tried to identify ''risk factors'' (Section 2.4); ''risk factor adjusted'' logistic regression was performed and the basic risk of elevated liver enzyme levels was estimated to be 4.9 and 2.2% in the verum and placebo group, respectively. The results were not easy to interpret, because some of the patients had a history of hepatitis and elevated liver enzymes before start of the treatment. In addition, it has to be taken into account that coumarin was administered as co-treatment with troxerutin, which may have a hepatoprotective effect (study findings from isolated perfused rat liver [24]). This would be a reason to expect a stronger hepatotoxic effect when coumarin is administered alone.

The published data is consistent with the existence of a subgroup of the human population that reacts sensitively to coumarin with hepatotoxic effects. If recurrent blood sampling is used to monitor liver enzyme levels, this group may amount to a single-digit percentage of the population, much higher than in drug responses often classified as ''idiosyncratic''. No clear-cut dose-dependent increase in severity of the effect was observed within the subgroup, although liver failure was observed only in patients treated with high doses (more than 100 mg daily). On the other

 

hand, people not sensitive to the effect evidently tolerate daily coumarin doses in the gram range. Drug-dependent moderate elevation of liver enzyme levels is often tolerated in clinical practice; if positive effects for the patient are expected, treatment is often continued with monitoring of the laboratory parameters. However, such risk-benefit considerations are not permissible for foods. These have to be safe in any case, and if dietary exposure to coumarin caused elevated blood liver enzyme levels, even if they were reversible, would not be acceptable.

### 2.3 Hazard assessment using human data

As outlined above, the frequency as well as the severity of coumarin hepatotoxicity in the human subpopulation is relevant, and the effect should be considered in hazard assessment. Due to underreporting, the cases known to the authorities cannot be used to estimate the frequency of these reactions, but they can be used to identify the lowest daily dose of coumarin able to cause hepatotoxicity. This dose can be used as a staring point for deriving a TDI.

In 1999, an expert opinion ''on the assessment of coumarin in medicinal products with regard to a hepatotoxic effect in humans'' was commissioned by the Federal Institute for Drugs and Medical Devices (BfArM) in Germany [25]. The 82 case reports (international notifications) of possible coumarin-associated liver damage available to the Institute at that time were evaluated from a pharmacological perspective. These reports included cases of liver failure (survival of the patients) and seven fatalities. A dose classification was possible for 51 cases from France, Ireland and Germany. The most frequent daily dose was 90 mg coumarin prescribed for the main indication ''lymphatic disorders and varicose veins''. Five cases (10%) occurred at the lowest doses (25 and 30 mg daily); of the three cases from Germany documented in more detail, two had developed hepatitis. According to the expert report, for part of the population liver damage cannot be ruled out at a daily dose of 25 mg coumarin.

In order to extrapolate from this effect level to a human NOAEL, a factor of 5 is considered justified in the case of a severe effect at the lowest observed adverse effect level. This results in a level of 5 mg coumarin per day, which is expected to cause no adverse effects even in sensitive subjects. When choosing this factor, it was borne in mind that knowledge on the mechanism of action in sensitive individuals is not available. As this group of persons must already be viewed as the most sensitive subgroup in the population, no additional intraspecies factor was applied. Using the established safe daily dose of 5 mg coumarin for an adult weighing 60 kg, a (rounded) TDI of 0.1 mg/kg body weight was derived by the Federal Institute for Risk Assessment (BfR) [5, 26]. This value based on human data agrees with, and lends support to the EFSA value based on animal data [4].

Case reports evaluated by Bergmann also allow an estimation of the time period critical for the onset of hepatitis in sensitive subjects [25]. The shortest periods documented were 5 days (dose: 90 mg daily), 16 days and 18 days (dose: 30 mg daily each). Therefore, it is not acceptable to significantly exceed the TDI over several weeks. The European Commission asked EFSA for an opinion on the relatively high cinnamon exposure during the Christmas season (Section 3.3), and EFSA concluded in 2008 that exposure to coumarin resulting in an intake three times higher than the TDI for 1–2 weeks is not a cause for concern [27].

### 2.4 What is the cause of coumarin hepatotoxicity in the human subgroup?

As outlined above, the varying susceptibility to hepatotoxicity of coumarin observed in animal species was attributed to the varying ability to detoxify the substance *via* the 7-hydroxylation pathway. In the human population, 7-hydroxylation of coumarin is catalyzed by a high-affinity CYP2A6 liver enzyme which is the only enzyme metabolizing this reaction in the human liver [28, 29]. Due to this predominant pathway, coumarin usually has a large first pass effect in the human liver after oral administration (94–98%) [30], leading to rapid urinary excretion of 7-hydroxycoumarin and its glucuronide. Using 5 mg coumarin as test substance to investigate the CYP2A6 phenotype, a mean total 7-hydroxycoumarin formation of 64% (range 20–100%) of the dose was observed in a European population of 110 volunteers; more than 95% of the metabolite formed was excreted within 4 h [31]. In subjects with high excretion rates of 7-hydroxycoumarin measured after oral coumarin administration, 0.13% of the dose or less was found as 3-hydroxycoumarin [32, 33]. In the same investigation, mean urinary excretion of *o*-HPAA (*via* 3,4-epoxidation) was found to be 3.5% (range 1.8–7.0%) of a dose of 1000 mg coumarin [34], comparable to the number of 4% (range 1–6%) of the dose of 200 mg coumarin reported earlier for eight volunteers [35].

The human data on rapid detoxification of coumarin is consistent with data of patients (Section 2.2) treated for weeks with high doses up to 7000 mg coumarin daily, corresponding to maximal doses of more than 100 mg/kg body weight daily (assumed weight 60 kg) tolerated without signs of hepatotoxicity [15]. Such a dose was lethal to two Beagle dogs within 9 and 16 days, respectively [12]. The apparently low susceptibility to the hepatotoxic effect of coumarin in the majority of the human population, as compared with many animal species, was attributed to detoxification *via* the 7-hydroxylation pathway predominant in humans. The ability to catalyze the 7-hydroxylation of coumarin was tested using liver microsomes from nine mammalian species. 7-Hydroxycoumarin was the major metabolite (greater than 70%) in humans and monkeys, but only a minor metabolite in rat (less than 1%), mouse (3%) and dogs (18%) [13]. According to these and comparable

 

results of other authors [3], it was discussed whether the interspecies factor used in risk assessment for extrapolation from animals to humans should be reduced [4, 8, 27].

However, as outlined above, a relevant subgroup of the human population is much more susceptible, as is most impressively demonstrated by the study of Loprinzi *et al.* [20] and with some limitations by the German study [22]. From the daily doses applied in these studies (about 6.7 and 1.5 mg/kg body weight, respectively, assuming a body weight of 60 kg) and from even lower doses (down to about 0.5 mg/kg body weight) in single cases reported to the authorities [25] it can be concluded that individuals of the human subgroup are more susceptible than various animal species investigated (NOAEL of 10 mg coumarin *per* kilogram body weight daily in Beagle dogs identified as most sensitive species [4]).

The cause of this higher susceptibility is unknown. Unfortunately, no phenotype data of coumarin metabolism is available from any patient affected by a hepatotoxic response following the treatment with coumarin. A genetic polymorphism of CYP2A6 as underlying mechanism has been discussed for a long time, possibly leading to an increased formation of 3,4-coumarin epoxide and *o*-HPA. This has been observed in a homozygous individual with an inactivating CYP2A6*2 allele: Following the oral administration of 2 mg coumarin, about 50% of the dose was excreted in the 8-h urine as *o*-HPAA, whereas 7-hydroxycoumarin could not be detected; the urinary metabolite excretion of the heterozygous parents was not found to differ from that of controls [36]. However, this is the only *in vivo* observation with documentation of alternative metabolism *via* the 3,4-epoxidation pathway in a CYP2A6–deficient subject. A very low or missing urinary excretion of 7-hydroxycoumarin after oral administration of coumarin was found in a significant proportion of Asians who lack the CYP2A6 protein completely due to the relatively high incidence of CYP2A6 gene deletion alleles in Japanese and Chinese populations. The frequency of poor metabolizers in Asian populations (up to 20%) is much higher than in Caucasian populations [37–39]. Unfortunately, the possible alternative routes of metabolism were not investigated in any of the studies of individuals identified as deficient in the 7-hydroxycoumarin pathway.

Therefore, it is currently unknown what CYP2A6 polymorphism with deficient 7-hydroxylation of coumarin means with respect to the 3,4-coumarin epoxide pathway and the possible generation of toxic metabolites. In addition to this lack of evidence, further observations do not support the assumption of a CYP2A6 polymorphism as cause of the higher susceptibility in the human subgroup:

(i) The vast majority of patients with hepatotoxic response following the treatment with coumarin is of Caucasian race; in this population, the frequency of poor metabolizers is close to zero [37]. In contrast, the two systematic placebo-controlled studies with recurrent blood sampling revealed a proportion of sensitive subjects in the single-digit range [20, 22]. In addition, CYP2A6 genotyping of 216 patients of the German study [22] revealed 7.4% subjects with defective genotype (CYP2A6*2 or CYP2A6*3 allele) all found to be heterozygous for the variant alleles; of the nine patients with elevated liver enzyme levels in the verum group, only one carrier of a variant allele was identified who exhibited an isolated γ-GT elevation without concomitant increase in transaminases. Additional genotyping of affected patients for the deletion of the CYP2A6 gene (CYP2A6*4 allele) revealed no further polymorphism. Therefore, no evidence was obtained that the polymorphism in CYP2A6 is a determinant of the coumarin-associated elevation of blood liver enzymes [21].

(ii) Rietjens *et al.* used a physiologically based toxicokinetic model to predict liver levels of the toxic *o*-HPA metabolite in rats and in human subjects with normal or deficient CYP2A6–catalyzed coumarin 7-hydroxylation phenotype; data from the literature as well as data obtained from *in vitro* investigations of microsomes was used to determine kinetic parameters for coumarin metabolism of animals and humans [40]. Modeling allowed the prediction of maximum tissue concentration of *o*-HPA in the liver of wild-type human subjects and of subjects deficient in 7-hydroxylation which were three and one order of magnitude, respectively, lower than the values predicted for rat liver. The authors concluded that even when 7-hydroxylation activity is missing, the formation of the hepatotoxic *o*-HPA metabolite will be significantly lower in the liver of humans than those expected in the liver of rats when exposed to a similar (low) dose per body weight. As the rat is a relatively coumarin-sensitive species [41] comparable to dogs (NOAEL about 10 mg/kg body weight) [12], it can be concluded that *o*-HPA is unlikely to be the toxic agent causing hepatotoxicity in the human subgroup which is more susceptible than rats and dogs, as outlined above.

From these observations it can be reasoned that CYP2A6 polymorphism with deficient 7-hydroxylation of coumarin is not the cause of the high susceptibility to hepatotoxicity in the human subgroup. Regarding other possible causes, previous hepatitis and elevated baseline γ-GT were identified as risk factors in the German coumarin study [22]. At least two disease states seem to reduce coumarin 7-hydroxylation. In patients with alcohol-induced liver disease, mean urinary 7-hydroxycoumarin excretion over 2 h was decreased in severe (18.0% of dose) and moderate (34.2% of dose), but not in mild (49.7% of dose) disease relative to controls (56.2% of dose) [42]. In previously healthy adult patients with acute jaundice from hepatitis virus A infection, mean total urinary 7-hydroxycoumarin excretion (0–8 h) was decreased by 37% compared with the values obtained from healthy volunteers [43]. However, the patients of the German coumarin study did not suffer from severe or acute liver disease, and

 

moderately decreased CYP2A6 activity levels are not expected to result in a strong increase of *o*-HPA liver concentrations (modeling data [40]). Therefore, the risk factors of previous hepatitis and elevated baseline γ-GT are probably not related to impaired coumarin detoxification.

Since evidence for a metabolic cause of the high susceptibility to hepatotoxicity in a human subgroup is missing, other possible causes have to be considered as well. Of the patients with hepatotoxic response described by Cox *et al.*, about half were re-treated with coumarin (after cessation of medication and return of elevated liver enzymes levels to normal) [16]. The authors observed that the time to onset of a rise in the liver enzymes was much shorter than in the first treatment period. In addition, they observed a favorable response (lower liver enzyme levels) to immunosuppressive therapy in three patients while on coumarin. These observations as well as the absence of any clear dose-dependency of the severity of response within the subgroup suggest an immune mechanism may be involved in the coumarin-induced hepatotoxicity in humans. To test for a drug-mediated allergic response, lymphocytes from the patients of the German coumarin study with elevated liver parameters were incubated with coumarin and 7-hydroxycoumarin; however, none of them showed a positive lymphocyte proliferation test response with a stimulation index of more than three [22]. In general, hepatotoxic responses observed during treatment are classified as ''adverse drug reactions'' which do not occur in most patients at any readily achieved dose of a drug and do not involve the known pharmacological effects of the drug [44].

## 3 Human exposure

### 3.1 Coumarin in cinnamon species

There has been no large-scale analysis of coumarin in foods. The substance is contained in various plants (for example sweet clover, tonka beans, lavender); however, given the general eating habits in most countries, these are probably not relevant for nutritional coumarin exposure. Recently, coumarin levels in specific plants or their essential oils were analyzed using a very sensitive method. Significant concentrations in plants relevant for food consumption were only found in cassia cinnamon (see below) and in woodruff (*Asperula odorata*, 203 mg/kg) [45]. The latter is used in Germany to flavor May punch (''Maibowle'') and has a long history of regulations due to its coumarin content. However, the alcoholic beverage is consumed by very few people and for only a short period each year.

Cinnamon bark is the dried inner bark of the shoots grown on cut stock of *Cinnamomum verum* J.S. Presl (Syn. *Cinnamomum zeylanicum* Nees, true cinnamon, Ceylon cinnamon) or of the trunk bark, freed of cork, of *Cinnamomum cassia* Blume (Syn. *Cinnamomum aromaticum* Nees, Chinese cinnamon, Saigon cinnamon, Vietnam cinnamon, Cassia cinnamon) [46]. The chemical composition of the two cinnamon species is different, particularly with respect to their coumarin levels. Coumarin concentrations were detected from below the detection limit to 190 mg/kg in Ceylon cinnamon ($n = 12$) and from 700 to 12 230 mg/kg in cassia cinnamon ($n = 12$) [47]. Due to these high concentrations in cassia cinnamon (compared with other foods), it seems obvious that – despite the relatively low amounts of the consumption of spices – coumarin exposure from food consumption is mainly due to cassia cinnamon. This applies to both the direct addition of cinnamon to foods but also the use of cinnamon oils by the food industry.

Cinnamon is available in the grocery store in dried form as cinnamon powder or as sticks; for the latter only, a visual differentiation between cassia and Ceylon cinnamon is possible. On the German retail market, mostly cassia cinnamon is available [48]; in the majority of cases, however, the botanical species is not indicated on the packaging. Analyses of cinnamon samples and of the most important cinnamon-containing foods done by the Federal States in Germany in 2006 and reported to the Federal Office of Consumer Protection and Food Safety up to March 2007 revealed the coumarin levels listed in Table 1. Some analyses done by the Chemisches und Veterinäruntersuchungsamt Karlsruhe were published in more detail [6]. With a median coumarin level of 2920 mg/kg (maximum 8790 mg/kg) the analyses confirmed the predominance of cassia cinnamon in the retail market. Coumarin levels in cinnamon-containing foods were found in the range expected from the recipes. For example, cinnamon star cookies (''Zimtsterne'') with the highest cinnamon content (about 1%, see also Section 3.3) were found to have the highest coumarin content (median 39.4 mg/kg, maximum 113.3 mg/kg). Most of these coumarin levels considerably exceeded the maximum permitted level (2 mg/kg) of the EU Flavorings Directive 88/388/EEC. Following the public discussion in Germany, most of the manufacturers took measures to reduce the coumarin content in 2007 and 2008.

### 3.2 Estimate of oral coumarin exposure

In its opinion of 2004, the EFSA calculated a theoretical added maximum daily intake (TAMDI) of 1.5 mg coumarin for an adult with a default body weight of 60 kg (0.025 mg/kg body weight per day) [4]. The calculation was performed considering the concomitant consumption of 324 g general beverages, 133.4 g general solid food, 27 g sweets, 2 g chewing gum and 20 g alcoholic beverages – each with the maximum level of coumarin permitted by the Flavorings Directive 88/388/EEC (2 mg/kg for foodstuffs and beverages in general). Lake calculated a TAMDI of 4.1 mg coumarin from general consumption data, which he rated as unrealistically high. A value of 1.2 mg coumarin (0.02 mg/kg body weight for 60 kg weight) seemed to him to be more realistic

 

**Table 1.** Coumarin levels (mean, median and maximum) in cinnamon and cinnamon-containing foods from the German market measured in 2006

| | *n* = | Mean (mg/kg) | Median (mg/kg) | Maximum (mg/kg) |
|---|---|---|---|---|
| Cinnamon[a)] | 170 | 2680 | 2920 | 8790 |
| Tea with cinnamon | 16 | 231.3 | 105.0 | 918.0 |
| Cinnamon star cookies (''Zimtsterne'') | 218 | 37.7 | 39.4 | 113.3 |
| Cereals with cinnamon | 28 | 25.5 | 23.9 | 60.0 |
| Almond cookies (''Spekulatius'') | 40 | 16.2 | 17.0 | 30.2 |
| Gingerbread cake (''Lebkuchen'') | 80 | 10.3 | 7.8 | 46.0 |
| Desserts with cinnamon | 29 | 10.2 | 10.8 | 19.0 |
| Chocolate with cinnamon | 25 | 9.4 | 5.6 | 32.9 |
| Mulled wine | 48 | 0.2 | 0.1 | 4.3 |

Analyses were done by the Federal states in Germany in 2006 and reported to the Federal Office of Consumer Protection and Food Safety (BVL) until March 2007. For all foods, minimum values were below the limit of detection.
a) Ground to powder in most cases (no differentiation between cassia and Ceylon cinnamon).

(assumption: maximum 5% of solid food flavored with cinnamon) [3]. These two worst-case estimates were based on high amounts of flavored foods consumed daily and on the maximum level of coumarin permitted by the Flavorings Directive 88/388/EEC. However, due to the predominant use of cassia, the majority of the cinnamon-containing foods in Germany were found to be far above this level, as shown by the data in Table 1. Therefore, the TAMDI approaches mentioned [3, 4] cannot be considered as reliable estimates at least for Germany.

Even with the improvements in knowledge on the coumarin levels in food (Table 1), it is still not easy to estimate the maximum daily intake of coumarin from cinnamon and cinnamon-containing foods. Epidemiological data on consumption of spices is inadequate. This is not only due to missing consumption data on particular spiced foods, but also due to a lack of estimates of the amount of spice used by the consumer at home (*e.g.* the use of cinnamon and sugar to spice rice pudding). In addition, a simultaneous dermal coumarin exposure may also need to be considered (Section 3.4). As an approach to estimate the seasonally higher consumption of cinnamon-containing foods during Christmas time, BfR has directed a telephone survey of adults (Section 3.3).

When estimating the consumption of cinnamon, young children require a separate consideration as a group with possibly high exposure on a body weight basis (higher food consumption due to their higher energy requirements and special eating habits). In this case, exposure data from the German VELS study is helpful (food consumption study to determine the dietary intake of infants and toddlers in order to estimate the acute toxicity risk from pesticide residues). For two non-consecutive 3-day periods, parents kept food records [49]. The evaluation of toddlers aged between 2 and 5 years ($n = 475$) showed that 140 children ate cinnamon or cinnamon-containing products at least on one of the six days recorded; 47% of the consumption days were between September and December. For these consumers the 97.5 percentile showed consumption of 0.22 g cinnamon per kilogram body weight (exposure on single a day, normally by eating rice pudding with cinnamon and sugar). Exposure lasting for longer periods was estimated by a worst-case approach assuming two of these meals per week. This results in a daily exposure of 0.063 g cinnamon per kilogram body weight per day. Based on a coumarin level of 3000 mg/kg cinnamon this would indicate a coumarin exposure of 0.19 mg/kg body weight as the worst case for oral exposure of the 2–5 year-old children [5].

A simple estimate of coumarin exposure in young children is possible using the levels measured in Christmas cookies (Table 1). For a 4-year-old child weighing 15 kg, a daily consumption of three cinnamon star cookies (''Zimtsterne'' weighing about 6 g each) with the maximum level measured already results in a coumarin exposure of 0.13 mg/kg body weight daily higher than the TDI (0.046 mg/kg body weight daily for the cookies with the median level). This calculation allows an estimation of the coumarin intake from single foods; however, other coumarin-containing foods may be consumed simultaneously. In order to get an estimation of the total coumarin intake during Christmas time (in adults), a telephone survey has been performed.

### 3.3 Telephone survey on cinnamon in Christmas-related foods

Christmas time is the period of highest consumption of cinnamon-containing foods. Typical Christmas treats like almond cookies (''Spekulatius''), gingerbread cakes (''Lebkuchen'') and cinnamon star cookies (''Zimtsterne'') have a long tradition in Germany as well as other European countries. In addition, a variety of teas, chocolates and desserts containing cinnamon have been launched in recent years to have a typical taste and flavor of winter and Christmas. In order to estimate the total amount of cinnamon consumed with Christmas-related foods by German adults, the BfR performed a questionnaire survey by telephone.

 

**Table 2.** Christmas-related foods in Germany asked for in the telephone interviews: estimated average cinnamon content, size of one portion, resulting content of cinnamon in one portion, and relative contribution of each food to the total cinnamon consumption of the interview sample.

| | Average cinnamon content (g/kg) | Size of one portion (g) | Cinnamon content in one portion (g) | Proportion of the total cinnamon consumption (%) |
|---|---|---|---|---|
| Tea[a)] | 77.1[b)] | 200 | 0.10[c)] | 22.2 |
| Mulled wine | 0.07[b)] | 200 | 0.02 | 0.9 |
| Dessert[a)] | 3.4[b)] | 150 | 0.52 | 4.4 |
| Chocolate[a)] | 2.8[b)] | 100 | 0.28 | 10.2 |
| Almond cookies (''Spekulatius'') | 5.8[b)] | 7.9 | 0.05 | 19.3 |
| Domino cookies (''Dominosteine'') | 0.5[d)] | 12.4 | 0.01 | 1.1 |
| Cinnamon star cookies (''Zimtsterne'') | 12.6[b)] | 5.8 | 0.07 | 5.2 |
| Small gingerbread cake | 3.2[b)] | 10.0 | 0.03 | 7.5 |
| Large gingerbread cake | 3.2[b)] | 50.0 | 0.16 | 9.8 |
| Home-baked cookies | 5.0[e)] | 17.0 | 0.09 | 19.4 |

a) Marketed as a winter/Christmas specialty.
b) Estimated from mean content of coumarin in Table 1, assuming a coumarin content in cinnamon of 3000 mg/kg.
c) Assuming the use of 2 g tea for a cup containing 200 mL and a coumarin transfer rate of 50% (average rate of transfer experiments performed by the BfR).
d) A ''Dominostein'' is a sweet primarily sold during Christmas season in Germany. On average one weighs 12.4 g, the base consists of 15% of soft gingerbread (manufacturer information). Next are a layer of jelly and a layer of either marzipan or persipan, it is covered with a thin icing of dark chocolate. Since there was no coumarin data available, cinnamon contents had to be estimated based on measurements of gingerbread cakes.
e) Basing on a list of cinnamon containing Christmas cookie recipes ($n = 30$), on average one cookie weighs about 17 g. Regarding these recipes cinnamon containing homemade cookie dough consists on average of 0.5% of cinnamon. The first question was about the number of consumed homemade cookies, and then the interviewee had to estimate how many of these cookies did contain cinnamon (34% of homemade cookies did reputedly contain cinnamon). If cinnamon proportion was not specified, this value was taken as computation base.

The survey was carried out by USUMA GmbH, an independent research institute for questionnaire surveys in Berlin. During three days of the third week of December in 2006, 1012 persons aged 14 years or older living in German-speaking private households were interviewed using a standardized questionnaire. The interviews lasted 12 min on average. The amount of selected foods was assessed by asking for ''the frequency of consumption within a typical Christmas week'' and typical portion sizes. To validate the estimates given by consumers for 1 week, a 24 h recall question was included for each of the selected food items. A plausibility check excluded two interviews because of implausibly high consumption of cinnamon-containing foods. The questionnaire also included socio-demographic parameters like age, gender and education. The sample was randomly generated and population-proportionally weighted to adjust for unavoidable biases. Hence the results are representative for the German population aged 14 years and older.

Interview partners were asked about their average consumption of ten different cinnamon-containing foods presented in Table 2 (which also shows the estimated average cinnamon content and portion size of each food item). To assess the individual coumarin intake, consumption data was multiplied by the mean coumarin levels of the foods in Table 1 (food monitoring data of 2006). In case of ''Dominosteine'' and ''home-baked Christmas-cookies'', only data on their cinnamon content was available; therefore, the coumarin content was estimated assuming an average coumarin content of 3000 mg/kg cinnamon as a rough estimate. In case of winter/Christmas specialty teas, the use of 2 g tea for a cup containing 200 mL and a coumarin extraction rate of 50% was assumed. Possible co-exposure to other cinnamon-containing meals eaten throughout the year (for example: rice pudding with home-used cinnamon or breakfast cereals with cinnamon) was not considered in the survey.

The total coumarin intake was estimated for each of the interview partners by adding up the intake data from the ten different Christmas-related foods. Figure 2 shows the distribution of all of the estimated individual consumptions per week, ordered by total amounts. As expected for spice-related consumptions, a high variation was observed: 42 people (4.2%) reported that they did not consume any of the 10 foods, whereas 154 people (15.2%) consumed half of the total coumarin amount. Mean coumarin intake was estimated to be 5.0 mg coumarin per week; median, 95th and 97.5th percentile were estimated to be 3.2, 15.9 and 21.6 mg coumarin *per* week, respectively. The coumarin intake of the heaviest consumers (six subjects) was estimated to be higher than 35 mg *per* week (maximally 47.5 mg *per* week). These consumers more or less reach an estimated daily coumarin intake of 0.1 mg/kg body weight (assumed weight 60 kg). These results confirm the often-used worst-case rule of thumb that the heaviest consumer may eat up to ten times more than the average consumer.

Regarding subgroups of the population, average coumarin intake was lower in women (5.0 mg *per* week) than in men (5.8 mg *per* week); this may be due to their lower general food and calorie intake. The age distribution

 

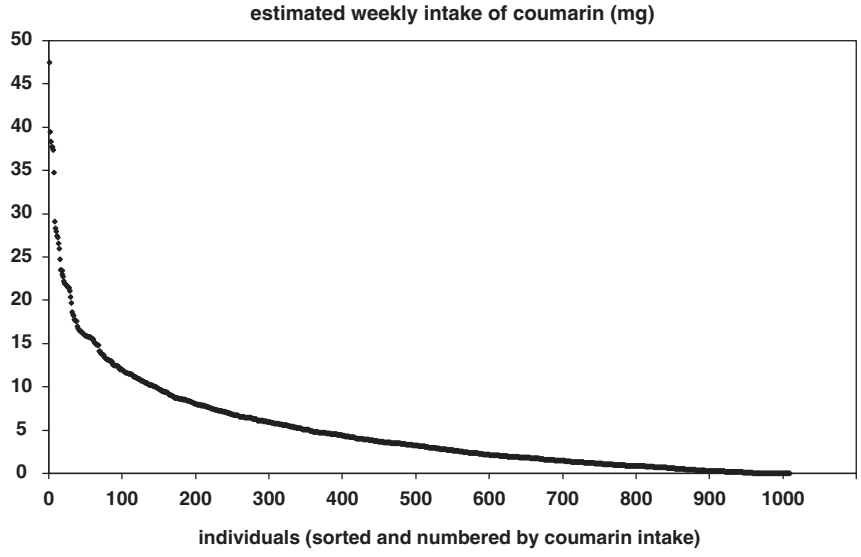


**Figure 2.** Individual weekly coumarin intake from Christmas-related foods in Germany in 2006, estimated from the telephone survey of 1012 consumers. The estimated intake of each individual subject is presented; subjects were sorted by their intake value.

showed a distinct trend towards a lower consumption at higher ages. Whereas the average intake of the group of 14- to 24-year-old persons was 8.4 mg coumarin per week, it was 5.0 mg *per* week in the group of 35- to 54-year-old persons, and 3.1 mg coumarin per week in the group of the more than 75-year-old persons.

As expected, the contribution of the ten different foods to the total weekly intake of coumarin in the interview sample was different (Table 2). The main contributions were from winter/Christmas specialty teas (22.2%), almond cookies (''Spekulatius'', 19.3%), home-baked cookies with cinnamon (19.4%) and gingerbread cakes (small and large size, 17.3%). Despite their relatively high coumarin content, cinnamon star cookies (''Zimtsterne'') accounted for only 5.2% of the total coumarin intake, as they only were eaten by a few people: nearly 80% of the interview partners reported not eating any of these cookies. This may have been influenced by the public discussion on coumarin in cinnamon and especially on cinnamon star cookies which took place in Germany in late 2006. Sixty-seven percent of the interview partners said they had noticed warnings in the media against a high consumption of cinnamon and cinnamon-containing foods.

The average consumption of cinnamon obtained from the telephone interviews was checked against market data from Germany. A total consumption of 2887 tonnes (amount imported minus amount exported) corresponding to an average consumption of 35.2 g cinnamon per year in Germany (population: 82 million) and a weekly average of 0.68 g cinnamon has been reported [48]. This number is lower than that of 1.7 g *per* week estimated from our study (assuming a coumarin content of 3000 mg/kg cinnamon). However, an important proportion of the yearly cinnamon consumption is expected to take place during Christmas time. If we assume that Christmas-related foods are offered intensively over a 10-week period, this would correspond to an average consumption of 17 g cinnamon or roughly half of the total annual cinnamon consumption during the Christmas period. This is considered plausible.

### 3.4 Dermal exposure

In addition to oral exposure, a simultaneous dermal exposure has to be taken into account as well. This is an uncommon case in risk assessment of food ingredients. In contrast to food production, coumarin is used without constraints as a fragrance in cosmetics, leading to a dermal exposure of consumers, which is by no means insignificant. The annual world production for the use as a fragrance in cosmetics today amounts to about 2000 tonnes [50]. Based on production data from the USA, an average daily coumarin amount of 1.2 mg per US American was calculated [51]. As the formulations of cosmetics are not normally made public, only insufficient data is available on coumarin levels. Lake referred to a compilation of the International Fragrance Association in Geneva, which indicates a coumarin level of 6.4% in a few thousand fragrance mixtures as the 97.5th percentile [3]. Based on this value he estimated a daily dermal exposure of 9.8 mg coumarin for adults (0.16 mg/kg body weight for a person weighing 60 kg) as a worst case. However, this amount appeared to be unrealistically high for him and he re-estimated a daily intake of 2.3 mg coumarin (0.04 mg/kg body weight for an adult weighing 60 kg) as reasonable worst-case scenario. This calculation reflected the fact that coumarin is absorbed quickly and efficiently *via* the skin (absorption rate approximately 60%: [3, 51]). Currently it is under discussion whether the risk of a hepatotoxic effect of coumarin from dermal uptake is comparable to that of from oral intake. Due to slower absorption and the fact that the first pass phenomenon does not apply, hepatic peak concentrations of

 

coumarin are expected to be much lower after dermal compared with oral exposure to the same dose. Accordingly, coumarin would be much less hepatotoxic after dermal compared to oral exposure, if hepatotoxicity is a threshold effect depending on the peak concentration, but not if hepatotoxicity is related to the area under the curve. The question of which dose metric is relevant, especially for the sensitive human subpopulation, is currently open.

## 4 Risk assessment

As is evident from the estimation of exposure, heavy consumers may reach a daily coumarin intake of 0.1 mg/kg body weight corresponding to the TDI just from the consumption of Christmas-related foods during autumn and winter. With an additional consumption of cinnamon, these consumers may exceed the TDI. Similarly, a heavy home-use of cinnamon as spice itself (for example rice pudding with sugar and cinnamon) may lead to an exposure in excess of the TDI during a relevant period of time, especially if the particular product is at the upper end of the range of coumarin content (possibly higher than 8000 mg/kg).

Therefore, no risk of hepatotoxicity of coumarin is expected for the vast majority of the population consuming low or moderate amounts of (cassia) cinnamon. However, consumers who really eat a lot may have a risk if they belong to the sensitive subgroup.

During the public discussions on coumarin and cinnamon in autumn 2006 in Germany, the question was raised whether coumarin in the food matrix of cinnamon has the same bioavailability as the pure compound used in animal experiments and human medicine. This issue was addressed by the German ''Senatskommission zur Beurteilung der gesundheitlichen Unbedenklichkeit von Lebensmitteln'' (SKLM) http://www.dfg.de/aktuelles_presse/reden_stellungnahmen/2006/download/sklm_natinh_en_05092006.pdf. [52]. It was concluded that in the absence of data on the influence of the respective constituent and the respective matrix in a foodstuff, toxicological data on the pure compound should be used as a basis for risk assessments. Clarification of the issue can only be achieved by further investigations on a case-by-case basis. Currently, the BfR is performing a human study to investigate the bioavailability of pure coumarin compared to coumarin in different foods containing cassia cinnamon.

Evidence for the possibility of a hepatotoxic response during high consumption of cassia cinnamon arises from a case reported to the BfR. In summer 2006, a 23-year-old woman was hospitalized with acute hepatitis. Laboratory tests produced no evidence of a viral infection. Liver biopsy showed distinct signs of inflammation, acute cholangitis and canalicular cholestasis. A toxic hepatitis was presumed, but anamnesis did not turn up any hints as to its cause. A slow but continuous recovery was observed in the following weeks. In autumn 2006, the patient became aware of the public discussions on cinnamon and hepatotoxicity. She remembered that as a big fan of cinnamon, during the 1–2 months before the onset of her hepatitis, she had consumed even higher amounts than before (about 1–2 g cinnamon every day, used to spice different foods). It is therefore suspected that this case was caused by high consumption of cassia cinnamon.

## 5 Summary and concluding remarks

Clinical data from patients treated with coumarin (case reports and controlled studies) revealed the existence of a relevant subgroup making up a single-digit percentage of the human population, which is sensitive for the hepatotoxic effect. In contrast to coumarin-treated patients, even slightly elevated liver enzyme levels, as a sign of liver damage, are not acceptable (even if reversible) in case of consumers eating food with a high coumarin content.

The underlying mechanism of coumarin-related hepatotoxicity in a human subgroup has not yet been elucidated. Considering all the data available, evidence for a genetic polymorphism of CYP2A6 with deficient 7-hydroxylation of coumarin as the cause of high sensitivity is missing. Especially investigations of coumarin metabolism pathways in patients with hepatotoxic response following the treatment with coumarin and in subjects with deficient 7-hydroxylation would be helpful to clarify the toxic mechanism. As long as this data is not available the question of a possibly metabolic cause for the hepatotoxicity in the human subgroup remains open, and other possible mechanisms have to be considered as well.

Evidence of coumarin hepatotoxicity in a subgroup of the human population is striking and has to be considered for risk assessment of coumarin, whatever the underlying mechanism may be. Considering the lowest dose (25 mg coumarin daily) with documentation of a hepatotoxic response in patients, a TDI of 0.1 mg/kg body weight daily was derived identical to the TDI derived by EFSA in 2004 using animal data [4]. For the latter derivation, an interspecies factor of 10 was used, as confirmed by EFSA in 2008 [27]. A reduction of this factor [53] from 10 to 2.5 (no kinetic sub-factor) was suggested by Felter *et al.* [8], based on the fact that – in contrast to humans – the CYP2A6 mediated detoxification to 7-hydroxycoumarin is only a minor pathway in many animal species including rodents and dogs. Their TDI of 0.64 mg/kg body weight daily, starting from of a NOAEL in rats of 0.16 mg/kg body weight daily [41], would be equivalent to an absolute dose of about 38 mg coumarin (assuming a body weight of 60 kg); for these doses, however, elevated liver enzyme levels and/or hepatitis is documented in sensitive people. Therefore, a reduction of the interspecies factor, based on kinetic data only, may be misleading in risk assessment, because a high susceptibility in a relevant human subgroup may be due to dynamic causes not

 

covered by an interspecies factor of 2.5 and an intraspecies factor of 10.

Comparison of the toxicological data on coumarin available from animals and humans on the other hand demonstrates that toxicological mechanisms may not be identical, and that in humans an important subgroup may be much more susceptible than the majority in the population. If available, data on toxicological effects in humans should be considered in risk assessment with high preference. Often such data is not available and risk assessment has to use animal data. In this case, a reduction of safety factors during extrapolation from animals to humans should only be made if this is securely justified.

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