Australian Government
Department of Health
Therapeutic Goods Administration

Safety review

Coumarin for use in topical listed medicines

Version 1.0, December 2019

TGA Health Safety Regulation

Contents

Abbreviations

ADI...Acceptable Daily Intake

ARTG.. Australian Register of Therapeutic Goods

bw..body weight

CE..coumarin-3,4,-epoxide

cm ....centimetre

EC..European Commission

EDI.. Estimated Daily Intake

EFSA.. European Food Safety Authority

FDA... Food and Drug Administration

g... gram

IFRA .... International Fragrance Association

IMAP ....Inventory Multi-tiered Assessment and Prioritisation

kg..kilogram

mg.. milligram

mL... millilitre

MoS...Margin of Safety

NICNAS ....National Industrial Chemicals Notification and Assessment Scheme

NOAEL...No Observed Adverse Event Level

NTP... National Toxicology Program

o-HPA.. *ortho*-hydroxyphenylacetaldehyde

o-HPAA.. *ortho*-hydroxyphenylacetic acid

PI .... Proprietary Ingredient

ppm...parts per million

RIFM .... Research Institute for Fragrance Materials

SUSMP..Standard for the Uniform Scheduling of Medicines and Poisons

TDI.. Tolerable Daily Intake

TGA..Therapeutic Goods Administration

Uf.. uncertainty factor

WHO..World Health Organization

/ .... per

1 Introduction

Coumarin is a naturally occurring organic chemical compound found in a number of food products, such as cassia cinnamon and tonka bean and is described as having a "hay-like" and "new-mown hay" odour (Clark, 1995). Commercially, coumarin is used for its fragrance properties and is estimated to be included in 90% of cosmetic and other fragranced products. In listed (low-risk) medicines, coumarin has been found in proprietary fragrance formulations included within products such as sunscreens, baby powders and nappy rash creams.

Listed medicines are self-selected by consumers and used for self-treatment. Under the TGA's regulatory framework, the low-risk associated with listed medicines is maintained by ensuring only ingredients that are demonstrably safe (and continue to be safe according to current evidence) be included within these products.

Coumarin is considered a Schedule 4 Poison (prescription only medicine) when used as an active ingredient and is currently available for use in listed medicines only as an active homeopathic ingredient when limited to a concentration of 0.001%. This 0.001% limit also applies to coumarin when included in certain herbal ingredients (such as *Cinnamomum* spp.).

However, coumarin (as a chemical ingredient) is **not** currently approved for use as an excipient in listed medicines. On 11 September 2018, there were 118 listed medicines identified by the TGA which contain the chemical ingredient 'coumarin' and are not compliant with TGA requirements, by virtue of containing an ingredient that is not approved for that use. Each of these medicines contain coumarin as an ingredient within a fragrance Proprietary Ingredient formulation ('PI').

A preliminary investigation into the safety of coumarin identified that there were potential safety concerns of hepatotoxicity, carcinogenicity and skin sensitisation. This review will consider these safety concerns and inform whether coumarin is appropriate for topical use in listed medicines in Australia.

1.1 Therapeutic use under consideration

As the chemical ingredient 'coumarin' is currently only included in listed medicines which are for topical administration, this review will only consider the safety of coumarin for topical use.

1.2 Identifiers and synonyms

Chemical Abstracts Service ID: 91-64-5

Accepted name: 2H-1-Benzopyran-2-one

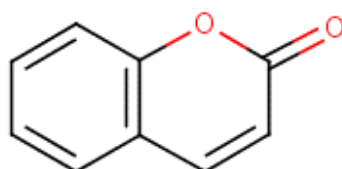Synonym: 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ-lactone

Common name: Coumarin

Figure 1.1: Chemical structure of coumarin (U.S. National Library of Medicine - Toxicology Data Network)

2 History and previous human use

2.1 Regulatory status

2.1.1 Australia

2.1.1.1 Use in prescription medicines

Prescription medicines containing coumarin as an active ingredient became available to the Australian public in July 1993 (Casley-Smith & Casley-Smith, 1995) as a treatment for lymphoedema. During the course of its use, 10 hepatotoxicity adverse events associated with coumarin (including 2 fatalities) were reported to the Australian Drug Evaluation Committee. Three years after general availability, the registration of coumarin-active medicines were cancelled (World Health Organization ['WHO'], 1996) and dermally-applied products were cancelled shortly thereafter.

2.1.1.2 Scheduling status

Currently, the Standard for the Uniform Scheduling of Medicines and Poisons ('SUSMP') lists coumarin "for therapeutic use (excluding when present as an excipient)" under Part 4, Schedule 4. Coumarin was included in Schedule 4 of the SUSMP following consideration of the Drug and Poisons Schedule Standing Committee in June 1991 (Meeting Number 61). The Scheduling of coumarin was considered on 3 further occasions since this time; however, these considerations were limited to the definition and effect of the qualifier within the entry referring to 'therapeutic use'. Under Schedule 4, Item 3(c) of the *Therapeutic Goods Regulations 1990*, this would preclude coumarin as an active ingredient in listed medicines above a concentration of 0.001% (Part 1, paragraph (2)(j) of SUSMP). Coumarin is available as an active ingredient in homoeopathic medicines; however, is not currently used in this way for any listed medicines.

Consideration of the SUSMP Scheduling resulted in an amendment to the Therapeutic Goods (Permissible Ingredients) Determination ('the Determination') in September 2017. This amendment required sponsors of listed medicines to declare the content of coumarin if those medicines contained the following active ingredients:

·   *Anthoxanthum odoratum*;

·   cassia cinnamon bark dry;

·   cassia cinnamon bark powder;

·   *Cinnamomum camphora*;

·   *Cinnamomum cassia*;

·   *Cinnamomum verum*;

·   cinnamon bark oil;

·   cinnamon dry;

·   cinnamon leaf oil;

·   cinnamon powder;

·   *Dipteryx odorata*;

·   *Galium odoratum*;

·   *Melilotus officinalis.*

2.1.1.3 Presence in listed medicines

On 11 September 2018, a search of the Australian Register of Therapeutic Goods ('ARTG') indicated that coumarin is an ingredient within 118 listed medicines, all with a dermal route of administration. Of these 118 listed medicines, 77 were sunscreen products and 10 were intended for use on babies or for the whole family (i.e. for treatment of rash).

Since the establishment of the Determination in 2016, before a PI can be granted availability for use in listed medicines, all ingredients within a PI formulation must be included in and meet the safety requirements for inclusion in the Determination; however, 81 fragrance PIs containing coumarin were granted listed medicines availability prior to this requirement. The concentration of fragrance PIs in listed medicines is limited to 1% or less of the final medicine. Historically, TGA did not frequently capture the quantities of the individual excipient ingredients within a PI. A single listed medicine may contain multiple fragrance PIs, leading to an unknown concentration of any particular PI excipient ingredients (such as coumarin) in the final medicine.

2.1.1.4 Presence in food and cosmetics

The Australia New Zealand Food Standards Code sets out a maximum coumarin concentration of 10mg/kg (0.001%) in alcoholic beverages (Schedule 19) and restricts *Cinnamomum camphora* and *C. micranthum* from sale as food due to coumarin being a natural toxicant (Schedule 24).

The National Industrial Chemicals Notification and Assessment Scheme ['NICNAS'] (n.d.) perfomed an accelerated assessment under the Inventory Multi-tiered Assessment and Prioritisation ('IMAP') framework for coumarins, and was not isolated to coumarin. This IMAP report concludes that coumarin be categorised as acutely toxic and the labelling and classification of coumarin should reflect the associated safety risks.

2.1.2 International

In the 1990s, coumarin was not only removed from therapeutic use Australia, but also in Belgium, France and Canada (Abraham *et al.*, 2010). A search of the Food and Drug Administration ['FDA'] (n.d.-a) online library for medicines indicates there are currently no coumarin-active medicines currently available.

Coumarin, as an isolate of *Cinnamomum aromaticum*, a synonym for *C. cassia*, is listed as a Natural Health Product in Canada (Health Canada, 2018).

The European Union (2009) requires leave-on cosmetic products to indicate the presence of coumarin if the concentration exceeds 0.001%, and rinse-off products at 0.01%. This requirement was considered and confirmed by the European Commission in 2012. The European Chemical Agency (n.d.) classifies coumarin as toxic.

The European Food Safety Authority ('EFSA') considered a Tolerable Daily Intake ('TDI') of coumarin (EFSA, 2004; 2008). EFSA considered carcinogenicity, interspecies differences in coumarin metabolism and the incidence of hepatotoxicity adverse events in human toxicity data. In 2004, based on hepatotoxicity in a two-year canine study, EFSA established a TDI of 0-0.1 mg coumarin/kg bw. EFSA maintained this TDI in 2008, with a concession that an exposure of 0.3 mg/kg bw for up to two weeks is not of safety concern. The European Regulations were changed in 2011 to take this into account, and the limit of 2 mg/kg coumarin in food stuffs from any source was increased to 50 mg/kg for traditional and/or seasonal baked good and 5 mg/kg for desserts (Bundesinstitut für Risikobewertung ['Bfr'], 2012). Food with any added coumarin is deemed adulterated and is prohibited from sale by the same agency (FDA, n.d.-b).

2.2 Exposure from all sources

2.2.1.1 Presence in food products

Coumarin is a naturally occurring component of a number of food products; including, but not limited to, Ceylon cinnamon, cassia cinnamon and tonka beans. Studies of the Norwegian (Fotland *et al.*, 2012) and German (Bfr, 2006) diet found that the EFSA TDI could be easily exceeded, with estimates of up to 1.2 mg/kg/day. The Canadian Food Inspection Agency (2016a, 2016b) examined the coumarin concentration of commercial foods and beverages. The Agency considered that the concentrations found did not cause concern for human health. A similar investigation occurred in the Czech Republic (Blahova *et al.*, 2011).

2.2.1.2 Presence in fragrance and cosmetics

Synthesised coumarin is a popular ingredient within fragrances, having been reported to be within 90% of fragrance compositions (Floc'h *et al.*, 2002) and is included in a standard patch test to screen individuals for fragrance sensitivity (Geier *et al.*, 2015).

2.2.1.3 Estimated exposure of coumarin

Lake (1999) estimated a combined fragrance (0.04 mg/kg/day) and dietary (0.02 mg/kg/day) total daily human exposure to coumarin at 0.06 mg/kg/day. The author of this paper is affiliated with BIBRA International, a toxicological consulting agency in the United Kingdom. The paper specifically mentions a range of dermally-applied products and does not reference sunscreens. It is considered that sunscreens are not a part of this estimation.

An Australia-wide telesurvey between 2007 and 2012 found that approximately 35% of respondents utilised sunscreen products as a form of sun-protection (Volkov *et al.*, 2013). Based on United Nations (2017) data, this would equate to potential exposure to 8.5 million Australians who are exposed to sunscreen products.

The [TGA risk management approach](#) specifies that products must be safe for their intended use and consideration of appropriate use of coumarin within listed medicine must factor in maximum dosage expected under recommended use. The Cancer Council (2017) recommends a teaspoon (5mL) for the face, neck and ears; a teaspoon for each arm and leg; and a teaspoon each for the front and back of the body at least every 2 hours. This amounts to 35 mL per application and, if an individual were to spend 8 hours a day in the sun, the recommended daily dose would equate to 140 mL of dermally applied sunscreen.

The amount of coumarin within some fragrance proprietary formulations used in listed medicines has not been advised to the TGA. Approximately 33% of the sunscreens in the ARTG have sufficient information to assess the coumarin concentration. According to the ARTG records obtained in September 2018, there was a maximum concentration of 0.01% coumarin in certain sunscreens; and a range of 0.025% to 0.000034% coumarin in the other listed medicines containing coumarin.

The maximum exposure to humans from Australian sunscreens with ARTG entries in which the coumarin content available is calculated in Equation 2-1. This figure is in addition to Lake's (1999) estimate of dermal exposure, which is 5 times lower. Moreover, this figure does not take into account the remaining 77% of coumarin-containing Australian sunscreens where insufficient data was available to assess coumarin content.

Equation 2-1: Maximal Estimated Daily Intake ('EDI') of coumarin from sunscreens where coumarin content is known

$$EDI_{sun} = \frac{(\text{applied volume})\times(\text{coumarin concentration})\times(\text{dermal absorption})\times(\text{coumarin density})}{(\text{body weight})}$$

$$= \frac{140 \text{ mL}^* \times 0.01\% \times 95\%^\dagger \times 935 \text{ mg/mL}^\ddagger}{60 \text{ kg}}$$

$$= 0.2 \text{ mg/kg}$$

Floc'h *et al.* (2002) estimated the maximal daily dietary intake of coumarin to be 11 mg. However, this estimate included a daily intake of 0.3 g cinnamon and calculated the coumarin content therein to be 10 mg (or 0.033 g/g). The Canadian Food Inspection Agency (2016a) examined 40 samples of cinnamon (of unspecified species origin) and reported an average coumarin content of 0.004 g/g, which reduces the coumarin associated with 0.3g of cinnamon intake to 1.2 mg (instead of 10mg). Applying this figure to the Floc'h *et al.* (2002) calculation results in a total estimated maximal daily dietary intake of 2.2 mg coumarin. Assuming a bodyweight of 60 kg, this would equate to 0.04 mg/kg/day. It is noted that the journal from which this article arises is not peer reviewed and the authors are representatives of a commercial fragrance company.

The German Federal Institute for Risk Assessment (BfR, 2006) estimated the total oral and dermal exposures for children at 2 to 5 years old to be considerably higher than adults. The higher exposure is attributed to cinnamon being more frequently included in the diet for children compared to adults, and that the body surface to body weight ratio of children is roughly twice that for adults.

3 Biological activity

3.1 Pharmacokinetics

3.1.1 Absorption

3.1.1.1 Dermal absorption

An *in vivo* investigation of male Sprague-Dawley rats found that the total absorption efficiency of dermally-applied coumarin in a hydrophilic ointment was greatly increased with a corresponding increase of application surface area (Ritschel & Hussain, 1988). This study found that 100% of the dose applied to the smaller application area was absorbed within 6 hours, and the total amount absorbed increased in a linear manner over the 6 hours. Concurrently, the blood concentration-time profile absorption of dermally-applied coumarin was found to more closely resemble that of intravenous administration, as opposed to oral. The author did consider the first-pass effect on coumarin metabolism in the context of these results (Ritschel *et al.*, 1979).

An *in vitro* examination of dermal penetration of coumarin through human skin found the dermis was the rate limiting factor (Ritschel *et al.*, 1989). A subsequent *in vitro* study of human skin compared the absorption of coumarin in an ethanol-based vehicle to that of an oil-in-water

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[*] Sunscreen daily usage discussed in 2.2 - Exposure from All Sources
[†] Total dermal absorption of coumarin in an oil-in-water emulsion (see 3.1.1 - Absorption)
[‡] (Lide, 2002-2003)

emulsion (Yourick & Bronaugh, 1997). A total absorption of 64.4 ± 0.29% was found with the ethanol vehicle and 98.0 ± 5.34% for the emulsion. In both cases, the absorbed dose was almost entirely accounted for at 6 hours following application. Testing occurred at 6-hour intervals over 24 hours.

In an investigation of dermal absorption in 3 human adult volunteers, a total absorption efficiency of 60% was found (Beckley-Kartey *et al.*, 1997; Ford *et al.*, 2001). The dose was delivered as a 0.2% concentration of coumarin in a 70% ethanol solution. Plasma concentrations of the dose increased in a linear fashion up to approximately 1 hour following application, at which point the concentration decreased in a linear fashion to almost 0 at 6 hours.

Sunscreens are required to include warning statements when the ethanol concentration is higher than 3% (Therapeutic Goods Administration, 2016). As at August 2018, approximately 11% of Australian sunscreens listed in the ARTG include this warning statement. As most Australian sunscreens are not ethanol based, it appears that the studies conducted with high concentration ethanol vehicles are not representative of the Australian sunscreens, rather, the absorption profile for coumarin in the oil-in-water emulsion (Yourick & Bronaugh (1997) is a more appropriate representation of Australian sunscreens considering the emulsion more closely resembles the formulation of sunscreens.

3.1.1.2   Oral absorption

Abraham (2011) considered whether the absorption of coumarin in humans via oral administration was effected by the delivery vehicle. A dose of 12 mg coumarin was administered to volunteers via 4 different oral dosage vehicles; cinnamon tea was considered to have resulted in the most efficient absorption (Table 3-1).

Table 3-1: Volunteers taking an equivalent doses of coumarin in differing deliver vehicles expressed significant differences ($p<0.005$) in total 7-hydroxycoumarin urinary excretion compared to chemically isolated capsules (Abraham *et al.*, 2011).

Vehicle	Total absorption
Isolated coumarin in capsule	62.8 ± 6.3%
Cinnamon in a capsule	56.0 ± 5.9%
Cinnamon tea	66.1 ± 5.5%
Cinnamon mixed in rice pudding	54.7 ± 5.6%

3.1.2   Distribution

Piller (1977) investigated the distribution of 3-^{14}C radio-labelled coumarin in female albino rats up to 100 hours following intraperitoneal administration. The blood, kidney, brain, heart, spleen, liver, lung, muscle, gut and skin were examined for coumarin concentration. Overall, the highest levels were found in the kidney and liver; closely followed by the gut. The total detected coumarin increased between 10 and 24 hours, and the author postulated that coumarin had been present in other, unexamined tissues. The overall half-life of coumarin and metabolites in examined tissues was about 43 hours. At 100 hours, 77.7% of the dose was accounted for in urine and faeces and 7.4% detected in the examined tissues, with the remaining 14.9% unaccounted for.

Lake *et al.* (2002) found rats treated with orally-administered 25 mg/kg bw coumarin retained 0.055±0.007%, 0.97±0.03% and 0.013±0.001% of the dose in the gut, liver and kidney respectively 96 hours after the initial dose. In the same study, hamsters with the same dose retained 0.027±0.003%, 0.51±0.07% and 0.013±0.001% in the same organs. In each of the models, greater than 98% of the dose was accounted for; and, comparable retentions were found in a 300 mg/kg bw dose treatment.

A further study exposed male Lister Hooded rats Ford *et al.* (2001) to a dermal application of 0.17 mg coumarin per rat and found 1.3% of the dose remained in the tissues at 120 hours. At this time point, 83.9% of the dose was recovered. However, the results provided do not indicate the distribution of coumarin and/or metabolites within these tissues.

Numerous review articles (Cohen, 1979; Fentem & Fry, 1993; Lake, 1999) and regulatory assessments (EC, 1999; WHO, 2000) have considered the available data on distribution of coumarin in human and animal models. The consensus is that tissue accumulation is insignificant in humans; however, these reviews and articles were largely concerned with an oral route of administration. The absence of first pass metabolism in a dermal route of administration would likely lead to an altered distribution profile, however this has not been investigated in the literature available.

3.1.3 Metabolism

Major differences are found in the metabolism of coumarin between primates (including humans) and other mammals (Cohen, 1979; Fentem *et al.*, 1992; Vassallo *et al.*, 2004). In primates, first pass metabolism has been found to efficiently (94-98%) convert coumarin to 7-hydroxycoumarin (Ritschel *et al.*, 1979; Ritschel *et al.*, 1988), which is readily excreted in urine (Ford *et al.*, 2001; Shilling *et al.*, 1969). However, the extent of 7-hydroxylation shows remarkable interspecies variation (Cohen, 1979; Hardt & Ritschel, 1983; Lake & Grasso, 1996; Ritschel & Hardt, 1983) and is considered to be a major contributing factor to the differences in hepatotoxicity between species (Abraham *et al.*, 2010; Farinola & Piller, 2007; Lake, 1999).

The 7-hydroxylation of coumarin is localised to the liver (Ritschel *et al.*, 1979) and is attributed to the action of the CYP2A6 enzyme (Pelkonen *et al.*, 2000). Rautio (1992) examined the efficiency of 7-hydroxylation in 100 volunteers and found 4 of the subjects had a reduced capacity compared to the rest of the group. It has since been found that polymorphism of CYP2A6 has been found to entirely suppress 7-hydroxylation in humans (Hadidi *et al.*, 1997) and frequency of non-wild type CYP2A6 have been reported in Chinese populations at frequencies up to 20% (Oscarson *et al.*, 1999). Reduction of CYP2A6 activity has been found in patients exhibiting infection (Pasanen *et al.*, 1997). It has not been possible to fully consider the link between CYP2A6 polymorphisms and coumarin-induced hepatotoxicity in this evaluation, as the polymorphisms have not been investigated in humans exhibiting hepatotoxicity (Abraham *et al.*, 2010).

Metabolism of coumarin preferring pathways other than 7-hydroxylation have been found in non-primate mammals (Fernyhough *et al.*, 1994; Vassallo *et al.*, 2004) and humans (Hadidi *et al.*, 1997). Pathways have been proposed including intermediates of coumarin-3,4,-epoxide (CE) and 3-hydroxycoumarin (Hadidi *et al.*, 1997; Lake *et al.*, 2002; WHO, 2000); however, there appears to be some disagreement as to the exact biochemical pathways involved.

Born *et al.* (2000) stated the CYP1A2 and CYP2E1 enzymes were involved in the metabolism of coumarin to CE; but an *in vitro* investigation by Zhuo *et al.* (1999) of mouse CYP2E1 resulted in an end-product of *ortho*-hydroxyphenylacetaldehyde (*o*-HPA). The conjugate product of CE and glutathione is readily excreted in the urine; however, *o*-HPA is a putative hepatotoxic agent and is considered to be another contributing factor in the greater propensity for coumarin-associated liver damage in rats (Felter *et al.*, 2006). Vassallo *et al.* (2004) reported a radically

reduced affinity for detoxification of *o*-HPA to *ortho*-hydroxyphenylacetic acid (*o*-HPAA) by rat hepatic cytosol compared to mouse and human liver cytosol.

Rietjens *et al.* (2008) conducted physiologically based toxicokinetic modelling to predict levels of coumarin and metabolites in liver microsomes with normal and deficient CYP2A6 activity. The accumulation of *o*-HPA in CYP2A6 deficient human samples was observed to be 70-500 fold higher than wild-type; however, the accumulation in rats was 1 and 3 orders of magnitude higher than deficient and wild-type humans respectively. The author suggested that the relative differences should be considered in extrapolation of rat toxicological data to human populations. This is discussed further in 4.3.1 - Tolerable Daily Intake.

3.1.4   Excretion

In all examined animal species, the major pathway of coumarin metabolite excretion is urinary. Lake (1999) examined 10 studies and summarised their data as per Table 3-2. The studies show that, with the exception the rat, at least twice the amount of coumarin-metabolites are accounted for in the urine compared to faeces. Few studies are reported as having considered the relative dose of coumarin detectable in the air expired by the test animals and, as such, it is difficult to make any confident conclusions relating to this route of excretion. The World Health Organisation (2000) stated that little to no biliary excretion is exhibited by humans following an oral dose of coumarin. This opinion appears to be supported for dermally-applied coumarin, as a study of 3 volunteers following a dermally applied dose of radio-labelled coumarin found 1.07±0.21% of the dose in faeces at 120 hours following administration (Ford *et al.*, 2001).

Table 3-3: Routes of excretion of single doses of coumarin in various species (Lake, 1999)

Species	Route of admin.	Dose	Collection time (hr)	% of Dose in		
				Urine	Faeces	Expired air
Mouse	Oral	200 mg/kg	24	46	-§	-
Mouse	Oral	50 mg/kg	24	67	-	-
Rat	IP	7.5 mg/animal	16	38	13	30
Rat	Oral	35 mg/kg	48	106	12	-
Rat	Oral	25 mg/kg	96	64	37	-
Rat	Oral	25 mg/kg	96	69	38	<0.7
Rat	Dermal	1 mg/kg	120	50	21	-
Rat	Oral	100 mg/kg	89 or 120	53	39	-
Hamster	Oral	25 mg/kg	96	90	12	-
Rabbit	Oral	25 mg/kg	96	90	12	-
Baboon	Oral	4 mg/kg	72	82	3	-
Marmoset	Oral	25 mg/kg	96	59	28	<0.7
Human	Oral	200 mg/person	24	82	-	-
Human	Dermal	2 mg/person	120	59	1	-

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§ '-' = Not examined

Shilling *et al.* (1969) accounted for 79% of an excreted dose as 7-hydroxycoumarin and a further 4% as *o*-HPAA within a 24-hour period from 8 volunteers. Meineke *et al.* (1998) investigated the *o*-HPAA urinary excretion of 30 volunteers following oral (2 and 1 g) and intravenous (250 mg) administration of coumarin. The results found in the orally-administered treatment groups that 5.13±3.71% and 3.52±1.67% of the initial dose was excreted as *o*-HPAA respectively, which are aligned with Shilling *et al.* (1969). The intravenous group excreted 1.52±1.21% *o*-HPAA. This is less than the oral groups; however, significance was not determined and the standard deviations were high. Hadidi *et al.* (1997) examined the coumarin metabolism of a human lacking CYP2A6 activity and 50% of an oral dose was found in the urine as *o*-HPAA; however, other metabolites were not identified.

4 Toxicology

4.1 Single-dose toxicity

The oral LD_{50} for coumarin is reported as 420 mg/kg bw in C3H/HeJ mice and 780 mg/kg bw in DBA/2J mice (Endell & Seidel, 1978). An oral LD_{50} in Carwoth rats was reported as 292-680 mg/kg bw (Hazleton *et al.*, 1956). The WHO (2000) assessment of coumarin presented that hepatotoxicity was reported in rats at 125-500 mg/kg bw and mice at 200 mg/kg bw (following both single oral and intraperitoneal administration), but not reported in Mongolian gerbils following intraperitoneal injection of 125-150 mg/kg bw (Fentem *et al.*, 1992). The same assessment took into account pulmonary toxicity reported in mice following oral gavage doses of 150 mg/kg bw and above (Born *et al.*, 1998).

There is no data available for coumarin regarding dermal or inhalation toxicity, nor skin or eye irritation.

4.2 Repeat-dose toxicity

Tanaka (2017) compared rats receiving a repeated dose (study duration of 28 days) of 200 mg/kg bw/day coumarin to a 200mg/kg bw single dose and found reduced hepatotoxicity presented in the repeated-dose group, and attributed the change to coumarin absorption and subcellular localisation of the CYP2E1 enzyme.

The available sub-chronic studies, detailed in Table 4-1, were designed by the study authors to inform the dosage of chronic studies which are discussed below. Rats administered coumarin by gavage of greater than 150 mg/kg bw/day exhibited nephrotoxicity. Rats and mice treated with the same dose presented with hepatotoxicity (National Toxicology Program ['NTP'], 1993).

Hepatotoxicity was exhibited during studies of chronic exposure to coumarin by mice, rats, dogs and baboons, but not hamster (Ueno & Hirono, 1981). The data presented for the hamster study does not provide for extrapolation of a dosage per kilogram bodyweight. Api *et al.* (2019) state that a No Observed Adverse Event Level (NOAEL) of 25 mg/kg bw/day was considered in a chronic rat study; however, adverse effects for stomach ulcers were observed at all dosages within males (NTP, 1993). As such the NOAEL stated by Api *et al.* (2019) is not considered to be appropriate.

Two other NOAELs were presented for orally administered studies, being 22.5 and 10 mg/kg bw in baboon (Evans *et al.*, 1979) and canine (Hagan *et al.*, 1967) respectively. The studies generally comply with the OECD Guideline for Chronic Toxicity Studies; however it is noted that the number of animals tested in both of these studies are lower than the minimum specified.

Additional chronic studies in mice and rats were considered that were not available for review, which were reported to have NOAELs of 150 and 50 mg/kg bw/day respectively (Research Institute for Fragrance Materials, 1983; 1984 as cited in Api *et al.*, 2019).

Table 4-1: Sub-chronic toxicology data

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (Gavage)	Rhône-Poulenc, Inc.	Mouse, B6C3F$_1$, 10 males and 10 females per group	91 days	0 mg/kg bw/day 19 mg/kg bw/day 38 mg/kg bw/day 75 mg/kg bw/day 150 mg/kg bw/day 300 mg/kg bw/day	2 males in high treatment died during week 1. 1 female died in week 2 in the 19 mg treatment, and another in week 12 in the 75 mg treatment (both attributed to gavage error). Anaemia in all treated animals. Liver weights of animals receiving ≥150 mg significantly greater. Centrilobular hepatocellular hypertrophy observed in 7 males and 7 females in 300 mg group.	N/A	(NTP, 1993)
Oral (Gavage)	Rhône-Poulenc, Inc.	Rat, F344/N, 10 males and 10 females per dosage	91 days	0 mg/kg bw/day 19 mg/kg bw/day 38 mg/kg bw/day 75 mg/kg bw/day 150 mg/kg bw/day 300 mg/kg bw/day	3 females and 1 male died during week 1 in high-dose treatment. Additional 2 males died during weeks 8 & 9 in same treatment group. Mean bw of males observed in 150 & 300 mg treatments reduced to 92 and 83% of controls respectively and bw of females in 19, 38 & 150 mg treatments increased to 107, 106 & 107% of controls (P<0.01). Incidence of centrilobular hepatitis and kidney damage in 150 & 300 mg treatments significantly different to control (P≤0.05). Significant dose related (75 mg and above) changes in erythrocyte: decrease in mean volume and haemoglobin, increase in count.	N/A	(NTP, 1993)

Table 4-2: Chronic toxicology data following oral application of coumarin

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (Diet)	Unknown	CD-1 mice, 52 per sex per group	100 weeks (male) and 108 weeks (female)	300, 1000 and 3000 ppm (equivalent to 45, 150, 450 mg/kg bw/day)	Lower bodyweight gain was reported during the first half of the treatment period for the mid and high-dose animals. No treatment-related effect on tumor incidence or type or any other histopathological changes were reported.	150 mg/kg bw/day	(RIFM, 1983 as cited in Api *et al.*, 2019)

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (Gavage)	Rhone Poulenc, Inc.	Mouse, B6C3F1, 70 males and 70 females per group	103 weeks	0 mg/kg bw/weekday	No significant effect on survival. Mean bodyweight of 200 mg dose lower throughout much of the study. Anaemia observed in 200 mg dose. Pathological observation of liver damage in female 200 mg dose and ≥100 mg male doses. Hepatocellular carcinomas observed in 50-100 mg dose females. Alveolar and bronchiolar carcinomas and pulmonary neoplasms observed in 200 mg dose group. Forestomach papilloma observed in 50 mg dose group.	Not established	(NTP, 1993)
				50 mg/kg bw/weekday			
				100 mg/kg bw/weekday			
				200 mg/kg bw/weekday			
		Rat, F344/N, 60 males and 60 females per group		0 mg/kg bw/weekday	Survival rate at 2 years for males with doses ≥ 50 mg approach zero. No significant effect on survival in other groups. Final mean bodyweight lower than controls for females of highest dose and all male test groups. Biochemical detection of hepatotoxicity in 100 mg dose and males only in 50 mg dose. Hepatic lesions in all test males and females of ≥50 mg dose. Biochemical and pathological observation of compromised renal function in all test groups. Forestomach ulcers detected in female 100 mg dose and all male test groups.	Not established	
				25 mg/kg bw/weekday			
				50 mg/kg bw/weekday			
				100 mg/kg bw/weekday			

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (Diet)	Unknown	Charles River CD SD-derived rats	104-110 weeks	333, 1000, 2000, 3000, and 5000 ppm (equivalent to 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)	Macroscopically, there was an increase in the incidence of liver masses noted among rats exposed to 5000 ppm. Increased liver weights were recorded in males and females of the 3000 and 5000 ppm groups and females of the 1000 and 2000 ppm groups. Microscopically, cholangiocarcinoma was reported among the rats of the high-dose group along with an increase in the incidence of cholangiofibrosis of the parenchymal liver cell tumors. A single incidence of cholangiocarcinoma in a male rat treated with 3000 ppm of coumarin was reported.	1000 ppm (equivalent to 50 mg/kg bw/day)	(RIFM, 1984 as cited in Api *et al.*, 2019)

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (Diet)	Not stated.	Rat, Osbourne-Mendel				0.0003% of dietary intake	(Hagan *et al.*, 1967)
		Not described	2 years	0 ppm of diet	Not described.		
		6 male & 6 female		30 ppm of diet	No effect.		
		5 male & 7 female		75 ppm of diet	Slight pathological damage to liver.		
		6 male & 6 female		150 ppm of diet	Growth retardation. Decrease in haemoglobin. Marked pathological damage to liver including cholangiofibrosis.		
Oral (Diet)	Tokyo Kasei Kogyo Co. Ltd.	Hamster, Syrian golden				Not established	(Ueno & Hirono, 1981)
		12 males & 12 females	24 months	Basal diet only	Food intake reduction of 20% in treatment groups between month 1 and month 5. No observed growth retardation. Poor survivability in 0.1% treatment. Tumour incidence significant in male 0.5% treatment only. No evidence of cholangiofibrosis or cholangiocarcinoma.		
		11 males & 13 females		0.1% Coumarin			
		11 males & 10 females		0.5% coumarin			
	Not stated	Dog					

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
Oral (capsule)		2 male & 2 female [Group 1]	297-350 days	10 mg/kg bw (6 days /week)	*No definite effect* according to study outcomes.	10 mg/kg bw/day	(Hagan *et al.,* 1967)
		2 male & 1 female [Group 2]	133-330 days	25 mg/kg bw (6 days /week)	Moderate emaciation and slight jaundice in one female. Weight gain in remaining dogs. Marked pathological distinctions found in liver compared to Group 1. Moderate haemosiderosis in spleen. Gall bladder moderately distended in two animals		
		2 male & 1 female [Group 3]	35-277 days	50 mg/kg bw (6 days /week)	Emaciation; slight to moderate jaundice in the two female dogs. One death at 35 days; remaining dogs sacrificed at 45 and 277 days. Marked pathological distinctions found in liver compared to Group 1. Spleen - large amount of haemosiderin. Bone marrow – pale		
		1 male & 1 female [Group 4]	9-16 days	100 mg/kg bw (6 days /week)	Male killed *in extremis* after 9 days. Female found dead on day 16. Marked emaciation, slight dehydration, and slight jaundice. Marked pathological distinctions found in liver compared to Group 1. Moderately pale spleen. Gastrointestinal - tract contents thick, dark, and tarry. Thin and fatty bone marrow. Moderately distended gall bladder.		
Oral (Diet)	British-American	Baboon					(Evans *et al.,* 1979)
		8 male	24 months	0 mg/kg/day			

Route	Formulation	Animal model	Treatment Period	Dosage	Outcomes (non-lethal toxicities, target organs)	NOAEL	Reference
	Tobacco Co. Ltd.	8 male	18 months	2.5 mg/kg/day	No observed effect on body weight gain within any group. Mean relative liver weight in 67.5 mg treatment significantly higher (P < 0.05) than control. Hepatocyte endoplasmic reticulum hypertrophy found in individuals within 67.5 mg treatment only. Mean enzyme activity and coumarin binding within liver found reduced in 67.5 mg treatment compared to other groups.	22.5 mg/kg/day	
		8 male	24 months	7.5 mg/kg/day			
		6 male	24 months	22.5 mg/kg/day			
		4 male	24 months	67.5 mg/kg/day			

4.3   Relative exposure

4.3.1   Tolerable Daily Intake

The WHO Food Additives Series (Joint FAO/WHO Expert Committee on Food Additives, 1981) was unable to determine an Acceptable Daily Intake ('ADI'), citing that further research into chronic exposure in the rat was required. The data requested by WHO was performed by the NTP (1993) and considered by EFSA (2004). The EFSA TDI of 0-0.1 mg coumarin/kg bw accounted for the human population susceptible to coumarin-related hepatotoxicity; this was done by utilising the NOAEL for canines, being 10 mg/kg bw/day. As canines exhibit reduced 7-hydroxylation capacity in comparison to primates and this pathway is considered to be critical in the rapid detoxification and excretion of coumarin in humans, the evaluator considers this to be well justified. EFSA calculated the TDI with uncertainty factors ('*Uf*') for interspecies and intraspecies variation. The canine study does not comply with the OECD Guideline for Chronic Toxicity Studies, and it is considered that a further *Uf* modifier may have been appropriate to account for the smaller sample size (International Programme on Chemical Safety, 1994). A further *Uf* modifier would result in a reduced TDI.

Felter *et al.* (2006) argues that an intraspecies *Uf* of 10 in consideration of a rat-extrapolated model is not justified as the o-HPA based toxicity issues are relevant to human metabolism. The ADI suggested by this study is 0.64 mg/kg bw and was based on a NOAEL of 16 mg/kg bw/day in rats (RIFM, 1984 as cited in Api *et al.*, 2019). The adjusted *Uf* was supported by Rietjens *et al.* (2008); however, these considerations were disputed by Abraham *et al.* (2010) on the grounds that the mechanisms of hepatotoxicity in the susceptible human subpopulation is not fully understood.

Similarly, in the calculation of a "Reference Dose", which appears to be a limit of tolerable exposure for coumarin for use in humans, Api *et al.* (2019) applied an *Uf* of 100. A NOAEL of 16 mg/kg bw/day was utilised, based on a rat study (RIFM, 1984 as cited in Api *et al.*, 2019, Carlton *et al.*, 1996 as cited in Felter *et al.*, 2006). Api *et al.* (2019) reported that within this study adverse effects were observed at 16 mg/kg bw/day and the selection of this lower NOAEL is not considered appropriate, and indicates that most appropriate NOAEL for extrapolating to human use is 10 mg/kg bw/day as specified by EFSA (2004).

Due to the lack of first-pass metabolism in dermal exposure and the hepatotoxicity concerns for coumarin, toxicological data specifically considering dermal application would be informative for consideration of coumarin as a fragrance in listed medicines. Such data was not available for review and the TDI proposed by EFSA presents the most robust assessment of acceptable use of coumarin available.

4.3.2   Estimated Daily Intake

In order to consider a safe level of dermal coumarin exposure when used in listed medicines, a concomitant consideration of dietary intake and intake from dermal exposure to non-listed medicine fragranced products is necessary. The available dietary exposure intake estimates discussed in 2.2 Exposure from All Sources, which are based on European and United States diets, are summarised in **Table 4-3**

Table 4-3: Estimated Daily Intakes of coumarin from dietary sources

EDI from diet (mg/kg bw)	Reference
0.19 (<5 years olds)	(BfR, 2006)
0.07	(EFSA, 2004)
0.02	(Lake, 1999)
0.04**	(Floc'h *et al.*, 2002)
0.01-0.06	(Fotland *et al.*, 2012)

Lake (1999) provided the only available estimate of coumarin intake from dermal exposure to other fragranced products, being 0.04 mg/kg bw.

4.3.3   Margin of safety

Combined dietary (Table 4-3) and non-listed medicine fragranced-product adult EDI of coumarin range from 0.05 – 0.11 mg/kg bw. The EDI of coumarin calculated from the available ARTG data (Equation 2-1) exceeds all available dietary EDI and is double the EFSA TDI. If a concentration limit of coumarin as an excipient in topical listed medicines aligned with the limit associated with coumarin as active ingredient (0.001%, see 2.1.1.2 Scheduling status), the maximal estimated daily intake arising from sunscreens would be reduced to 0.02 mg/ kg bw and, combined with dietary and non-listed medicine fragranced product EDI, approximates the EFSA TDI.

4.4   Genotoxicity & carcinogenicity

Carcinomas have only presented in animal models at doses higher than 100 mg/kg bw/day (Abraham *et al.*, 2010). The European Union considered the available data on the genotoxicity of coumarin over a series of panels (EC, 1999; EFSA, 2004) and considered studies in compliance with Option 1 of the [TGA adopted guideline](#) for genotoxicity studies. (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2011).

In addition, to published studies on mutagenicity (NTP, 1993), unscheduled DNA synthesis (Edwards *et al.*, 2000) and an *in vivo* micronuclease test (Api, 2001) the panels considered a further unpublished study which determined that coumarin does not covalently bond with DNA in the liver or kidney and in 2004 (EFSA) determined that the tumourogenicity of coumarin is preceded by toxicity in the target organ; which provided the basis for determining an ADI based on toxicological data.

4.5   Developmental and reproductive toxicity

There were no primary sources available for review considering the reproductive or developmental toxicity of coumarin; however, review articles that consider such data are discussed below. Literature reviews by the WHO (2000) and United States NTP (1993) cited limited sources in relation to reproductive or developmental toxicity of coumarin. The Australian National Industrial Chemicals Notification and Assessment Scheme (n.d.) considered the same sources, and determined that the limited information available to them did not provide for a conclusion to be drawn on the reproductive or developmental toxicity of coumarin. Api *et al.* (2019) describes developmental and reproductive toxicity studies not available for review;

** Adjusted as discussed in 2.2 Exposure from All Sources

there is insufficient information to assess whether the studies comply with international guidelines for such investigations (OECD, 2016).

Roll and Bar (1967 as cited in Api *et al.*, 2019) conducted a developmental toxicity study in NMRI mice fed diets containing 0%, 0.05% 0.1% and 0.25% coumarin. Stillbirths between control group and 0.1% and 0.05% coumarin diet groups were not significantly different; however, the 6.1% still birth rate in the 0.25% coumarin group was significantly higher than control. Analysis of Caesarean sections found the 0.25% coumarin diet group contained significantly reduced bone ossification of fetuses 18 days *post coitum*. The NOAEL for the developmental toxicity of coumarin was reported to be 0.1%, and extrapolated to 150 mg/kg bw/day; however, the basis for this calculation was not described in the report. Developmental toxicity was not reported for studies with a mixture of coumarin and rutin conducted in rats, rabbits, or miniature pigs; however, administered dosages were not described (Grote *et al.*, 1977 as cited in Api *et al.* 2019).

A multi-generation reproductive toxicity study was reported by Preuss-Ueberschar *et al.* (1984 as cited in Api. et al, 2019) in Wistar rats exposed to a therapeutic product containing a combination of 15 mg coumarin and 90 mg troxerutin. Twenty-three male and 46 female rats were administered (via oral gavage) 0, 1-, 8-, 64- and 128- fold of the daily therapeutic doses for humans prior to, and during mating phases. Adverse events relating to parental fertility, fetal deformity rates or postnatal developments of pups were not observed up to the highest dose. The highest dose is extrapolated to approximately 96-192 mg/kg bw/day; however, the basis for this calculation was similarly not described by the author.

4.6 Local tolerance

The skin sensitisation properties of coumarin are well established. The European Commission (2012) reports that coumarin is an allergen of special concern, as between 100 and 1,000 cases of reaction have been published. Available studies are summarised in Table 4-4. The frequency of coumarin sensitisation is of such concern that it is included in Fragrance Mix II, a patch testing kit to determine if commercial fragrance constituents are a causal factor in dermatologic conditions (Bennike *et al.*, 2017). The inclusion of coumarin in this mix may not be sufficient to detect all coumarin-sensitive patients, as numerous cases of sensitivity to coumarin have been reported in patients insensitive to the fragrance mix (Bennike *et al.*, 2017; Mutterer *et al.*, 1999).

Table 4-4: Summary of available skin sensitisation data in humans

Coumarin conc. (%)	*n*	Result	Method	Reference
8	25	No positive reactions	Kligman (1966) maximization test	(Greif, 1967)
2.5	367	10 positive reactions	Patch test (Schnuch *et al.*, 2008)	(Krautheim *et al.*, 2010)
5	50	No positive reactions	Patch test (North American Contact Dermatitis Group, 1984)	(Emmons & Marks, 1985)
5	5735	8 positive reactions	Patch test	(Bennike *et al.*, 2017)
ND††	1021	9 positive reactions	Patch test	(Goolamali *et al.*, 2009)

†† 'ND' = Not disclosed

Some reports have been made that the sensitisation effect associated with coumarin is a result of coumarin-derivatives present in impure ingredients and not to pure coumarin (Pan *et al.*, 2014). In consideration of this issue, the EC (2006) noted that there was insufficient data on the purity of coumarin in the European market to re-consider any coumarin restrictions. In addition, the EC cited an article which recorded 2 patients with sensitivity to a 2% solution of 99.9% pure coumarin. The available data does not provide any data on coumarin purity in the Australian market, and currently no default standard applies which could define acceptable coumarin purity.

Fragrance manufacturers often rely on publications of the International Fragrance Association ('IFRA'). The current IFRA standard (2009) recommends that the usage of coumarin in fragrances be restricted due to sensitisation effects, with concentration limits of 0.1% in products for children and 1.6% for body creams[‡‡]. The standard claims a No Expected Sensitisation Level (NESL) from an exposure of 3.5 mg/cm^2 coumarin on human skin, i.e this amount is not expected to induce skin sensitisation. The proposed NESL is greater than the amount of sunscreen exposure to the skin when testing the Sun Protection Factor for sunscreen products (ISO 24444); as such sunscreens used according to directions to use would not exceed the proposed NESL.

4.6.1   Phototoxicity

Addo *et al.* (1982) state that 5% coumarin in paraffin was found to induce photosensitivity dermatitis in 3 out 50 volunteers. Api *et al.* (2019) considers that coumarin is not expected to present a concern for phototoxicity or photoallergenicity at concentrations up to 8% in other vehicles.

5   Human studies & adverse reactions

Numerous reports of hepatotoxicity have been reported in humans following orally-administered coumarin at therapeutic (25 mg and over) dosages (Andréjak *et al.*, 1998; Anon., 1997; Bassett & Dahlstrom, 1995; Beinssen, 1994; Casley-Smith & Casley-Smith, 1995; Loprinzi *et al.*, 1997; Morrison & Welsby, 1995).

Coumarin was removed from therapeutic use by the TGA in 1996 following the report of 10 adverse events (including 2 fatalities) associated with oral administration of the drug, during 3 years of general availability. A dose-dependent relationship to the frequency of coumarin-associated hepatotoxic events has not been established (EFSA, 2008). Cox *et al.* (1989) calculated a frequency of hepatotoxicity events related to therapeutic doses of coumarin to be 0.37%.

A 23-year-old woman was hospitalised due to acute hepatitis in 2006. A medical history failed to reveal a cause of the inflammation. The patient became aware of a relationship between cinnamon and hepatotoxicity; and reported that her (cassia) cinnamon intake had increased to 1-2 g cinnamon daily in the 2 months preceding the onset of symptoms (Abraham *et al.*, 2010).

An additional report was received by the TGA in relation to an admission in 2016 of a 60-year-old male presenting with acute hepatitis with underlying liver synthetic impairment. Cause was not apparent following blood tests, however, the patient described taking teaspoons of cinnamon daily. Following literature review, the reporter attributed the cause to cinnamon.

These reports suggest that deviation from the EFSA established ADI of 0.1 mg/kg bw has the potential to cause hepatotoxicity. In addition, it can be inferred that medical professionals did

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[‡‡] The current IFRA standard for coumarin indicates the document should be reviewed in 2013. However, an updated standard was not available.

not initially consider non-therapeutic exposure to coumarin when attempting to determine causality of hepatotoxicity.

As excipient ingredients are not required to be advertised on medicine labels, any potential adverse events related to dermal excipient-coumarin exposure would be impossible to correlate by consumers or medical professionals.

6 Regulatory considerations

Therapeutic use under consideration

The safety of the chemical ingredient 'coumarin' for topical use in listed medicines.

Regulatory status

Coumarin is currently not available as an active therapeutic ingredient in Australia or the United States. Legislative requirements specify that coumarin as an active ingredient may not be present within listed medicines at concentration greater than 0.001%. Concentration restrictions are currently in place for coumarin in Australian alcoholic beverages at 0.001%, European general foodstuffs at 2 mg/kg, and it is entirely prohibited from addition to United States foodstuffs. European cosmetics are required to provide label warnings for leave-on products containing coumarin in concentrations above 0.001%, and rinse-off products at 0.01%.

Exposure

Coumarin is present in foodstuffs and is a staple component of many artificial fragrances. Oral exposure has been estimated at levels of 0.02 to 1.2 mg/kg bw/day and dermal exposure from non-sunscreen fragranced products estimated as 0.04 mg/kg bw/day. Children have been reported as receiving a greater level of exposure to coumarin than adults from both dietary and topical sources. As per section 4.3 above, maximal estimated exposure to coumarin from sunscreens in adults is 0.2 mg/kg bw/day (based upon ARTG products where coumarin concentrations are known).

Combining the Cancer Council (2017) recommended directions for use of sunscreens for the Australian environment and the maximum known coumarin concentration (0.01%) within listed medicines, the maximal estimated exposure of coumarin from sunscreens (0.2 mg/kg bw/day) is five-times higher than that estimated for other topically applied fragranced products combined. In comparison, sunscreen products with a coumarin limited to the level specified for use as an active ingredient (0.001%) would deliver 0.02 mg/kg bw/day.

Coumarin is already included in multiple listed medicines as a fragrance and may be in 90% of other fragranced commercial products, often undeclared on the product and at levels unknown to the TGA and the Australian public.

Biological activity

Total absorption of coumarin in sunscreen-like products has been reported as 95% (Yourick & Bronaugh, 1997). The absorption kinetics of topically-applied coumarin are indicated to be a linear profile up to a peak at between 1 and 6 hours. The highest concentrations of coumarin and its metabolites have been found in the liver, kidneys and gut; however, tissue accumulation is not considered to be of concern.

The most common metabolic pathway (7-hydroxylation) of coumarin in humans is considered to be highly efficient and of no toxicological concern, and coumarin is rapidly hydroxylated and excreted through this pathway. However, in as-yet undefined population sub-sets, genetic polymorphisms result in a greater reliance on less efficient pathways, such as 3,4 epoxidation. These less efficient pathways are suspected to be the causal factor of hepatotoxicity in susceptible populations, but this has not been established.

Toxicology

The TDI for coumarin is 0.1 mg/kg bw, as established by EFSA (2004) and is based on the NOAEL from a chronic oral-exposure study in canines, being 10 mg/kg bw/day. Estimations of European daily dietary intake make up 20-1200% of the oral ADI. European foodstuffs have had coumarin concentration restrictions for some time, which were relaxed after these estimates were calculated. As such, these estimates are considered to be conservative in relation to current intake. No estimations have been prepared based on an Australian diet, where there are no restrictions applied to coumarin content in foodstuffs other than alcoholic beverages.

The complex interaction between oral and topical routes of administration for coumarin make it difficult to establish an ADI (and corresponding concentration limits) for topical administration which factors in dietary exposure. The maximum recommended daily dose of a sunscreen containing coumarin at 0.001% would allow for a dietary and cosmetic intake of 0.08 mg/kg bw without exceeding the TDI.

Genotoxicity & carcinogenicity

Any carcinogenic activity would be preceded by toxicity in the target organ and therefore the carcinogenicity potential of coumarin is not considered to be a significant concern. Carcinogenicity of coumarin is considered to not be via a genotoxic mode of action.

Developmental and reproductive toxicology

Studies were discussed by WHO (1996) and Api *et al.* (2019), who proposes a NOAEL of 150 and 96 mg/kg bw/day for developmental and reproductive toxicology respectively.

Local tolerance

Coumarin is an established skin sensitiser. Of the data available to the TGA, sensitisation is exhibited at the lowest concentration studied (2.5%). Coumarin-derivatives within an impure coumarin-ingredient have been suggested as the causal factor; however, this has not been conclusively established. Api *et al.* (2019) proposes a sensitisation threshold of 3.5 mg/cm^2, which is not expected to be delivered by sunscreen products. There is some evidence to suggest phototoxic potential of coumarin; however, this has not been exhibited in vehicles other than paraffin.

Human studies & adverse events

Hepatotoxicity presented in 0.37% of human volunteers in clinical trials of orally-administered coumarin conducted in Australia. When considered in combination with the estimated 8.5 million sunscreen users in Australia (see section 2.2), this equates to roughly 30,000 susceptible individuals. One case of acute hepatitis has been attributed to oral consumption of cassia cinnamon. There have been no reported adverse events in literature or to the TGA associated with dermal coumarin exposure. The number of patients treated with topically-applied coumarin, as an active ingredient, was significantly less than oral products; and in the context of coumarin as fragrance, fragrances are often not stated on product labels, and therefore more difficult to identify and associate with the occurrence of an adverse-event.

Summary of thresholds proposed to addressed safety concerns

The safety concerns described above and the proposed thresholds at which these concerns are appropriate are summarised in Table 6-1.

Table 6-1: Summary of proposed thresholds for safety concerns

Safety concern	Proposed threshold
Chronic exposure	10 mg/kg bw/day
Genotoxicity	Not required
Carcinogenicity	Not required
Development toxicology	150 mg/kg bw/day
Reproductive toxicology	96 mg/kg bw/day
Skin sensitisation	3.5mg/cm^2 topical application[§§]
Phototoxicity	Greater than 8% (other than paraffin)

7   Conclusions

Evaluation of the currently available data suggests the risk-benefit profile is not favourable for unrestricted topical use of coumarin as a fragrance in listed medicines, due to the safety concerns and the lack of data for key safety considerations.

Safety concerns regarding chronic exposure to coumarin, including hepatotoxicity, remain outstanding. The proposed threshold to mitigate these concerns is lower than the thresholds proposed for developmental and reproductive toxicology, skin sensitisation and phototoxicity (Table 6-1). A maximum limit of coumarin in topical products which mitigates the chronic exposure concerns will also mitigate the risk of these other concerns.

The proposed threshold which addresses chronic exposure data was robustly considered in the establishment of a TDI (0.1 mg/kg bw) by EFSA (2004). The absence of a reliable estimation of Australian intake of coumarin from dietary and cosmetic sources suggest that a conservative maximum daily exposure is appropriate to avoid exceeding this TDI. As a maximum recommended daily exposure from a sunscreen containing 0.001% coumarin would make up 20% of the TDI, topical listed medicines and sunscreens should not exceed this concentration.

The safety of coumarin within listed medicines intended for use in children is not supported due to the likely increased exposure to coumarin from diet and body surface area-to-weight ratio (BfR, 2006). As such, a warning statement to that effect should be specified on the labels of listed medicines containing coumarin.

[§§] This is greater than the specified dose of 2 mg/cm^2 for sunscreen testing according to ISO 24444

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