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THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE WORKING WITH NATURAL AROMATIC MATERIALS

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Coumarin: The Real Story (Update 2: Feb. 2009).

Compiled from a number of Cropwatch sources.

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[See also Cropwatch's Coumarin Bibliography in this series].

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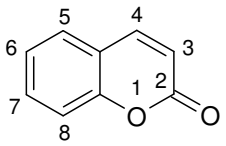
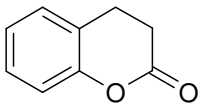
1. *What is it?*

Coumarin (2H-1-benzopyran-2-one) CAS No 91-64-5, is a crystalline white solid when seen pure, with a hay-like, sweet aromatic creamy odour with certain nutty shadings, much used in synthetic form as a fragrance ingredient for perfumes and for fragranced soaps and detergents. Coumarin has a widespread occurrence in natural products too (see separate section below), and is a representative of the *lactones* class of chemical substances (where a lactone is an ester group integrated into a carbon ring system).

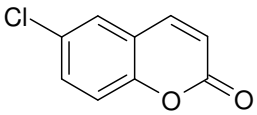


coumarin

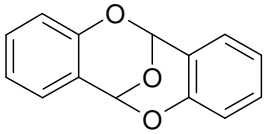
In contrast to natural coumarin, two commercial synthetic coumarin samples manufactured via synthesis from *ortho*-cresol, were found to have 19 impurities >1ppm (Vocanson *et al.* 2006), the main substances identified including 3,4-dihydrocoumarin, 6-chlorocoumarin and 12-epoxy-6H-12H-dibenzol[b,f]-1,5-dioxacin. The authors identify all 3 materials as being moderste sensitizers. Trade associations (including COLIPA) have tried to belittle the finding that pure coumarin in not an allergen, by stating that the situation of coumarin's non-allergy may only apply to (pure) Rhone-Poulenc derived material. Unfortunately however, many dermatological studies have been carried out with synthetic coumarin of unknown purity, and it is perfectly possible that impurities are responsible for the weak alleged allergenicity of coumarin.



3,4-dihydrocoumarin



6-chlorocoumarin



12-epoxy-6H-12H-dibenzo[b.f][1,5]dioxocin

Coumarin was also used in food flavourings, but use has ceased because of worries about potential hepatotoxicity (Hazleton *et al*. 1956; Hagan *et al*. 1967). EFSA has currently recommended a limit of 0-0.1mg/d coumarin, by itself or in conjunction with cimetidine is used to treat malignant melanoma & metastatic renal cell carcinoma (Marshall *et al*.1994), and prostatic carcinoma (Mohler *et al*., 1994) – all through Zhuo *et al*. (1998).

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Zhuo X., Gu J., Zhang Q.-Y., David C., Spink D.C., Kaminsky L.S. & Ding X. (1999) "Biotransformation of Coumarin by Rodent and Human Cytochromes P-450: Metabolic Basis of Tissue-Selective Toxicity in Olfactory Mucosa of Rats and Mice" *Pharmacology & Exptl Therapeutics* **288**(2), 463-471.

2. *Recent developments,*

a.) EFFA Plays Games with 'The Schnuch Evidence'.

To recap, 26 alleged allergens, 16 occurring in natural complex substances, were identified in SCCNFP Opinion 0329/00 (rubber stamping previous IFRA-RIFM

information) and passed into EU legislation under the Directive 2003/15/EC, amending Directive 76/768/EEC. The criteria for the inclusion of these materials as allergens by the SCCP has never been satisfactorily explained (Storrs 2007). The legislation requires a labeling obligation for finished cosmetic products containing any of the 26 identified allergens present at 0.01% in products rinsed off the skin, or 0.001% in leave-on products,

The July edition of the German consumer magazine *Öko-Test*, reported on studies done by the IVDK, an information network association of dermatologists, headed up by Prof. Schnuch. It concluded that not all the 26 allergens identified by SCCNFP Opinion 0329/00, and enshrined in the 7th Amendment to the Cosmetics Act, bear the same risk, and criticises the EU Commission for treating them all as equal. Regarding coumarin, the report concluded the risk of being an allergen was too small to consider. Schnuch *et al.* (2007) report in a further study conducted in four periods of six months from Jan 2003 to Dec 2004, on the frequency of sensitisation to the 26 allergens. The authors conducted the patch-testing studies with a large number of consecutive, unselected patients with suspected allergic dermatitis to these 26 compounds. Schnuch *et al*. concluded that for some of the alleged allergens amongst the 26, neither restriction nor labeling seem justified, and that EU regulators should review the previous decisions taken.

In a totally uncharacteristic move, EFFA (2008) contacted DG-Enterprise asking for labeling requirements for **10** allergens listed under Directive 2003/15/EC to be reconsidered. These were: benzyl alcohol, benzyl benzoate, methyl heptine carbonate, hexyl cinnamal, anisyl alcohol, linalool, benzyl salicylate, amyl cinnamal, limonene & g-methyl ionone. This was apparently in the light of the evidence of Schnuch *et al*. (2007) identifying these materials extremely rare sensitizers, and in 3 cases, not sensitizers at all. However Schnuch *et al* identified **11** substances as rare sensitizers – EFFA missed out coumarin. The reason for this may just possibly be that EFFA was acting as messenger for IFRA, and IFRA were about to include coumarin under their new set of restricted substances, under their new standards announced in their 43rd Amendment (see below),

In 2008, Schnuch addressed the IFRA Workshop - *Allergy Prevalence in Fragrance*, November 4, 2008, Brussels, Belgium, and reiterated his previous contentions "There are obviously fragrance ingredients among the 26 which are, with regard to contact allergy, of great, others of minor, and some of no importance at all" - coumarin being one of them. Annette Orloff, of DG Enterprise, who mentioned the 2004 labelling legislation connected to the notorious 26 allergens situation, on the other hand, was reported (Weller 2008), somewhat disturbingly we feel, as outlining her happiness to work with the trade-sponsored IFRA organisation, and mentioned the 2008 revision of the Cosmetic Directive where 74 of IFRA-restricted ingredients have been directly imported into Annex III. I guess if you have an important regulatory job in Brussels, and an industry-funded organisation like IFRA hands over safety policies and supporting data on

a plate, I guess you would be happy to work with them. But don't confuse this cosy situation with what should happen in a democracy. .

b.) IFRA Restrictions under its' 43rd Amendment.

As we stated in *Cropwatch Newsletter* Aug 2008, we (Cropwatch) are fairly sure in our own minds that pure coumarin is not an allergen, but that impurities in certain commercial grades may be responsible for its misdiagnosis as one. Further Vocansen *et al*. (2006) found that pure coumarin does not imitate cell proliferation in the LLNA procedure, whereas common impurities do. Whether natural coumarin is an allergen, where it occurs in natural products (see below) is, as far as we can establish, presently unclear.

In spite of controversy over the evidence for the classification of coumarin' as an allergen, as far as we can see IFRA have been no help whatsoever in defending the future of this key perfumery ingredient. The 43rd IFRA Amendment Standard restricting coumarin seems to be based on 4 unpublished studies between 4 and 6 years old, on which the Standard is supposedly based, so the general public is none the wiser about the robustness of the studies, their relevance to the present state of knowledge of allergenicity in 2009, nor about the source and purity of coumarin - factors which have proven absolutely crucial in previous experimental work. Amongst the crumbs from the table offered to the general public by IFRA on their website is REXPAN's conclusion that the No Expected Sensitisation Induction Level (NESIL) for whatever grade(s) of coumarin were used in the experiment(s) was 3,500µg/cm^2.

RIFM (Research Institute for Fragrance Materials, Inc.), 2003. Local Lymph Node Assay with coumarin. Unpublished report from Rhodia Services, 26a May. Report number 47072. (RIFM, Woodcliff Lake, NJ, USA).

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RIFM (Research Institute for Fragrance Materials, Inc.), 2005. Human Repeated Insult Patch Tests with coumarin. RIFM report number 47274, January 6 and 6b. (RIFM, Woodcliff Lake, NJ, USA).

c.) Federal Institute for Risk Assessment Gives Coumarin Warning[1].

The Federal Institute for Risk Assessment (BfR) recently maintained in a feature dated 20.12.07 on their website (BfR 2007) that they had "evaluated the analytical results of the controlling bodies of the federal states in order to assess the scale on which cosmetics contribute to consumer exposure to coumarin", They considered that consumers could already exceed the Tolerable Daily Intake (TDI) of coumarin, which they quoted as 0.1mg/Kg (European Food Safety Authority, EFSA), just by using cosmetics with high coumarin levels They found that "it has not been fully elucidated whether coumarin taken in via the skin has a

similarly harmful effect on the liver to coumarin ingested from the gastro-intestinal tract". If the BfR were aware of the scientific literature on the subject, they might have cited the paper by Yourick & Bronaugh (1997) who found that coumarin rapidly penetrated rat & human skin and is not metabolised by enzymes in the skin. Applied coumarin in fragrances & cosmetics is thereby presumed to rapidly enter the systemic circulation to be metabolised by the liver. Prof Andreas Hensel, President of the BfR, recommended that coumarin should not be used in cosmetic products for infants & toddlers as a precautionary measure.

That last statement, you might think, has come 140 years too late. Coumarin has been extensively used in fragrances including those for infant care products to Cropwatch's certain knowledge, since the commencement of its commercial production in 1876, and infant toxicity has not been revealed to be a problem thus far. The Industrieverband Körperpflege und Waschmittel e.V. (IKW) does not agree however (IKW 2008), stating that fragrances in cosmetics currently on the (err…German?) market for infants contain below 0.0001% coumarin (<1ppm). Further the IKW conclude that the maximum levels of coumarin in certain product categories ***assumed*** by BfR in its consideration constitute "very rare exceptional cases." Strange, we thought the website article had said the BfR hadn't assumed anything, but had "***evaluated the analytical results***". Finally the IKW state that "there are robust scientific indications that the hepatotoxic effects of coumarin observed after oral intake are not to be expected from intake through the skin." (but references for this assurance not provided).

Lake (1999) in a detailed review of coumarin metabolism, toxicity & carcinogenicity, found the intake of coumarin from combined diet & cosmetic sources to be 0.06 mg/day, and that coumarin intake is safe at 100 times this figure. Lake (1999) also states that this exposure level is over 2000 and over 3000 times lower, respectively, than those which produce liver tumours in rats (quoting Carlton *et al*. 1996) and lung tumours in mice (quoting NTP, 1993). Doses of coumarin of 8 to 7000 mg/day for 2 weeks to 2 years have been given in therapeutically to lymphoedema & liver & lung cancer patients, & with cimetidine in anti-neoplastic treatments (Lake 1999). However human hepatotoxicity has been observed as a result of these therapeutic interventions according to EFSA.

At least one perfumery organisation has commented internally to its members (late 2007/early 2008) that Prof. Hensel has not understood species differences relevant to coumarin metabolism (see below). IFRA has also posted a statement on its' website on this issue (still there Jan 2009!), claiming to speak for the Fragrance Industry, & dismissing any toxicity risk from dermal absorption of coumarin from fragrances. Interesting that IFRA should make a statement apparently endorsing IKW's pronouncements on low coumarin levels in fragrances, when Gruenwald (through Lake 1999) quoted an IFRA survey which showed the high average coumarin level of 6.4% in thousands of analysed perfumes. True, it's not absolutely clear whether this figure represents cases where coumarin is present in the perfume, rather than all perfumes (i.e. if used,

its there at an average of 6.4%, rather than the more unlikely proposition that all perfumes contain coumarin at 6.4%). It is also possible that in the meantime, things could have changed. But it seems, 'though, that IFRA possibly need to think about employing a Continuity Editor for some of their more rushed statements such as this, as some of us have long memories.

Cropwatch, being an independent organisation, can take a broader overview: If coumarin is, or has been, employed in fragranced cosmetic products intended for babies & infants at moderate to high concentration levels (as it certainly has been in cosmetic products for adults), we don't know for sure that detoxification mechanisms in babies/very young children are exactly similar to, or as efficient as, those operating in adults. Secondly, the actors above may not have been aware of the human genetic polymorphism concerning coumarin metabolism such that not all humans metabolise coumarin exclusively via the safer 7-hydroxylation route - there some may a proportion of 'low 7-hydroxylators' (see for example Hadidi *et al* 1997) who may be more at risk to coumarin exposure.

To elaborate the point above, it could be that all humans use a proportion of other metabolic paths, other than the major 7-hydroxylation route, in order to detoxify coumarin. The scheme of Lake (1999) shows the following metabolites: 3-, 4-, 5-, 6-, 7- and 8-hydroxycoumarin (3-HC, 4-HC, 5-HC, 6-HC, 7-HC and 8-HC), o-hydroxyphenylacetaldehyde (*o*-HPA), *o*-hydroxyphenylethanol (*o*-HPE), o-hydroxyphenylacetic acid (*o*-HPAA), *o*-hydroxyphenyllactic acid (*o*-HPLA), *o*-hydroxyphenylpropionic acid (*o*-HPAA), *o*-coumaric acid (*o*-CA), dihydrocoumarin (DHC), 6,7-dihydroxycoumarin (6,7-diHC) and 4-hydroxydihydro-coumarin-glutathione conjugate (4-HDHC-GSH conjugate).

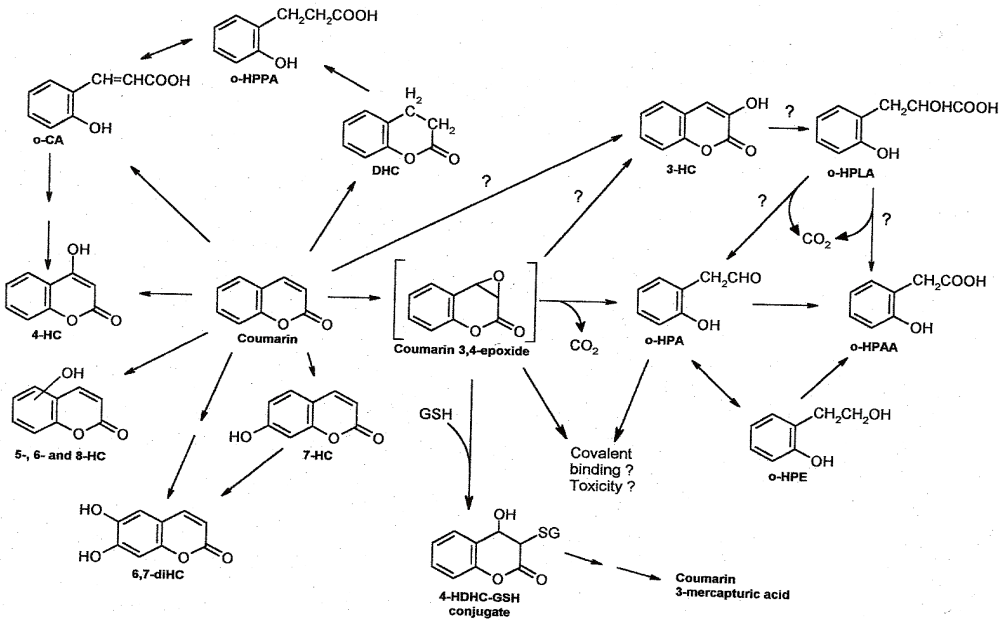

Fig (i) Some pathways of coumarin metabolism after Lake (1999): based on Born *et al*. (1997); Cohen (1979); Fentem *et al*. (1991); Lake *et al*. (1992a,b), Norman and Wood (1984).

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) re-considered coumarin toxicity in the 'ninties, with especial regard to genotoxic potential, looking at the possibility of DNA-coumarin adducts in the liver & kidney of rats. The found no evidence for this. The Panel further concluded that coumarin's liver toxicity is not directly correlated to 3,4 coumarin epoxide / ortho-hydroxy phenyl acetic acid (*o*-HPPA), but the ratio of bioactivation: detoxification, & this is the consideration hat probably dictates species susceptibility to coumarin-mediated hepatotoxicity.

Taking account the possibility of genetic polymorphism over coumarin metabolism in humans, which may negate the major 7-hydroxycoumarin route in favour of certain other routes, EFSA endorsed the AFC Opinion on coumarin, informing that "the overall NOAEL for liver toxicity in the most sensitive animal species, based on hepatotoxicity in a two year dog study, was 10 mg coumarin/Kg bw/day. Applying a safety factor of 100, a TDI of 0 - 0.1 mg coumarin/Kg bw can be established."

There are some indications that this limit is now considerd too severe, and more research is needed to more properly assess the risks. As it is it is still a matter of judgment how precautionary we need to be on restricting coumarin levels in cosmetics - obviously a harsh coumarin limit would severely affect not only the types of perfumes that could be sold (i.e. traditional fougères) but also limit the use of a number of essential oils & absolutes.

One further item for consideration is contained in published paper (Givel 2003), where the author paints a different light on the public availability of toxicological information relating to coumarin toxicity in tobacco perfumes. Continued use of coumarin in tobacco perfumes until 10 or 20 years ago demonstrates the conflict between a duty to protect the health of the people of the nation, against the right to keep trade secrets (i.e. the breakdown of tobacco fragrance formulations). Givel reports that "despite known severe toxic and carcinogenic risks to humans (in cigarettes), coumarin was also reportedly used as an additive in pipe tobacco in the USA at least as late as 1996" (and in cigarettes supposedly in 1985). This is in complete contrast with the ban on coumarin addition to foodstuffs on health grounds.

[1]Adapted from an Aromaconnection blog by the author, to be found at http://www.aromaconnection.org/2008/01/coumarin-again.html

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Schnuch A. (2005) *Öko-Test*, No. **7** (July) 2004, 55

Schnuch A., Uter W., Geier J, Lessmann H. & Frosch PJ. (2007) "Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature." Contact Dermatitis **57**(1), 1-10.

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Vocanson M., Goujon C., Chabeau G., Castelain M., Valeyrie M., Floc’h F., Maliverney C., Gard A. & Nicolas J.F. (2006) “The Skin Allergenic Properties of Chemicals may depend on Contaminants” *Int Arch Allergy Immunol* **140**, 231-238

d.) The NTEF’s campaign against the coumarin-containing *Angel* perfume[2].

The Las-Vegas based NTEF (National Toxic Encephalopathy Foundation) has received some media attention over allegations that Clarins *Angel* perfume (Thierry Mugler) contains toxic ingredients causing (amongst others) ocular damage. It appears that NTEF president Angel de Fazio had filed a lawsuit in the District Court in Clark County Nevada against Clarins in October 2004 claiming that one spray of the company's *Angel Parfum* had left her permanently disabled (Montague-Jones 2007b). The same report reveals that, again allegedly according to Clarin's legal spokesperson, de Fazio's claims had been dismissed in January 2007, the court ruling "that the allegations were without merit and brought in bad faith. She was ordered to pay Clarins $77,851 in costs, fees and sanctions".

Earlier, Montague-Jones (2007a) had reported that the NTEF had stated that *Angel Parfum* fits the FDA definition of a health hazard because it contains the scent coumarin, which it considers to be a dangerous poison. The Aromaconnection blog (www.aromaconnection.org) also reported the story on 12th Nov 2007, informing that the FDA has accepted a petition from the National Toxic Encephalopathy Foundation (NTEF) to have *Angel Perfume* declared "Misbranded", and had asked that its importation be halted until the issue is resolved. Nina Immers posted a long comment to this carried item (Immers 2007) reeling off some well-known toxicology studies on the potential hazards associated with the uncontrolled use of perfumery ingredients. Searching for any comments relevant to *Angel* perfume, there is a statement that coumarin is hepatotoxic in mice because it is metabolised to coumarin 3,4-epoxide, which has been linked to tumour formation in rats, and it is claimed that there is evidence that this metabolic route occurs in humans. Although Cropwatch was initially doubtful about the validity of this point, we do think that this area needs to be clarified by further research, to examine whether there is a quantifiable risk to the small number of people that cannot detoxify coumarin by the 7–hydroxycoumarin route, as mentioned elsewhere in this document.

Cropwatch had some previous correspondence with de Fazio in Jan 2007, where we commented that the analysis of *Angel* perfume available at http://www.national-toxic-encephalopathy-foundation.org/ocular test0001.pdf. was extremely poor and that perhaps Cropwatch could have provided a more comprehensive analysis[3]. We also asked if any the components allegedly responsible for ocular damage caused by *Angel* perfume had been identified (actually stated as damage to the cornea), Angel de Fazio indicated (at the time) that this information was not available.

[3]You could take the view that *Angel* by Thierry Mugler (Clarins) has the elements of a chypre fragrance (patchouli-evernyl accord), but more importantly is perhaps the first ground-breakingly successful "gourmand" fine fragrance. It is very sweet, having chocolate, red berry, praline, vanilla & cassis aspects. In a comprehensive analysis, you might expect to find veltol, patchouli, evernyl, hedione, frambinone, vanillin, canthoxal, a cassis base and certain lactones present.

Cropwatch was uncertain why *Angel* perfume had been particularly identified for criticism since there are fragrances still currently available arguably with higher coumarin contents - for example the original "Joop Homme" (Joop 1989) or "Le Male" (Jean Paul Gaultier 1995). Further, an NTEF press release on Aug 27th 2007 seemed to confuse the actual ingredients in *Angel* perfume with allergens required to be disclosed by labeling. A further NTEF news release of 27th Oct 2007 by de Fazio that maintaining that coumarin is a harmful perfumery ingredient is supported by Jack D. Thrasher, Ph.D., who is described as a "Toxicologist/Immunotoxicologist/Fetaltoxicologist". Unfortunately almost all the supplied references supposedly supporting the case do not actually concern coumarin at all - they concern ***coumarins*** such as warfarin, so Cropwatch can't see this feature actually contributes anything to the case. Nevertheless Montague-Jones picked up the NTEF suggestion that coumarin affected prenatal development & reported it in *Cosmetic-Design* (Montague-Jones 2007c), & subsequently the article was circulated to the membership of several perfumery trade organisations. We believe this story, which has nothing to do with coumarin, originates from two studies by Wesseling *et al*. (Wesseling *et al.* 2000; Weisling *et al*. 2001) which refer to pre-natal etc. exposure studies to the oral anti-coagulants acenocoumarol, phenprocoumon & coumadin (Warfarin™) ("***coumarins***"), and acenocumarol & phenprocoumarol respectively, all of which are capable of crossing the placenta and may affect the central nervous system. Coumarin however is not an anti-coagulant (Feuer 1974).

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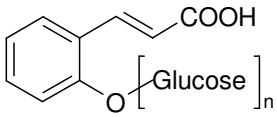
To summarise, Cropwatch believes that the NTEF haven't (yet) made a convincing case that coumarin is a dangerous perfumery ingredient presenting quantifiable risks to cosmetic users.

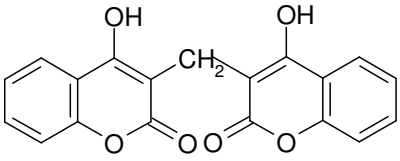
[2].Adapted from the author's blog posting on www.aromaconnection.org

3. *Coumarin-containing Natural Products.*

Coumarin occurs widely in natural products, generally being liberated from the corresponding glycoside (melilotoside) on drying coumarin-containing herb material. Dicoumarol is a microbiological biotranformation product in spoiled Melilotus Clover and other hay products, and its presence in fodder at >10ppm is cause for concern, as it is responsible for fatalities by hemorrhaging in cattle. This is because dicoumarol interferes with vitamin K reductase in the liver and the liver is unable to reactivate vitamin K, which leads to a decrease in vitamin K-dependent clotting proteins. The study of this compound paved the way to the discovery of anti-coagulant drugs such as warfarin.



melilotoside



dicoumarol

Coumarin occurs widely in natural products; the following natural aromatic materials are of note:

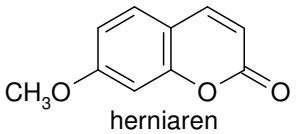
Some Natural Coumarin Sources	**Notes**
Anthoxanthum odoratum L. **Flouve oil**	Both essential oil and absolute produced.
Carphephorus odoratissmus (J.F. Gemel) syn *Liatris odoratissima* Mich. syn. *Trilisia odoratissima* (J.F. Gmel.) Cass. **Deer tongue or Liatris**	1.6% coumarin. Ratio of coumarin: dihydrocoumarin: 2,3 benzofuran in volatile fraction of extract 1:3:20 (Appleton & Enzell 1971).
Cinnamomum cassia J. Presyl. **Cassia oil**	Coumarin 4-11% (Burfield 1999). Coumarin to 8.73% (TNO 1996) Eu Pharm V (2) allows 1.5 to 4.0% coumarin in cassia oil monograph.
Cinnamomum zeylanicum **Cinnamon bark & leaf oils**	To 0.3%; rarely to 0.7%.
Dipteryx odorata (Aubl.) Wild. & sometimes *D. oppositifolia* . **Tonka bean absolute**	1-3%, or up to 10% coumarin in tonka beans (Hagers Handbuch 1973); also dihydrocoumarin, *o*-coumaric acid, ethyl & methyl meliotate etc (Ehlers *et al.* 1996). Tonka absolute contains up to 65% coumarin
Galium odoratum L. syn. *Asperula odorata* **Woodruff absolute & concrete**	Variable; coumarin content develops on drying herb, although headspace of freshly cut woodruff found to be 80% coumarin (Surburg *et al.* 1993). Used in alcoholic beverage flavourings (e.g. vodka).
Hierochloe odorata (L.) Beauv **Sweet grass**	Use to flavour vodka in Russia.
Lavandula spp. **Lavender & Lavandin qualities**.	Lavender absolute to 8.0% coumarin; lavandin absolute to 5.0% coumarin. Spike lavender oil to 0.3% coumarin.
Lolium perenne L. & other spp. incl. *Phleum pratense* (Timothy grass), *Poa pratensis* L. (Meadow grass), *Cynosurus cristatus* (Crested Dog's-Tail), *Anthoxylum odoratum* L. and *Melilotus* spp. **Foin oil**	Essential oil and absolute produced. Foin essential oil contains some 8% coumarin.
Melilotus alba Medik. **Bokhara Clover, or White Sweet Clover**	Less used than Common Melilot (q.v.)
Melilotus officinalis L. (Pallas) **Common Melilot or Yellow Sweet Clover**	0.9% coumarin on dry weight basis. Wagner (1996) says 0.25-0.45% coumarin in herb, together with umbelliferone, scopolin etc.
Mentha spp. **Peppermint oil**	20 ppm (TNO 1996)

Table 1: The occurrence of coumarin in some common herbs & natural products.

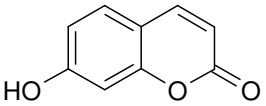
Coumarin also occurs in trace amounts in the oils/extracts of:

Billy Goat Weed ***Ageratum conyzoides*** L.
Sweet wormwood ***Artemisia annua*** L.
Mugwort ***Artemisia vulgaris*** L..
Carrot Seed oil ***Daucus carota*** L. ssp ***sativus*** (Hoffm.) Arcang.
Champaca ***Michelia champaca*** L.,
Narcissus spp**.**
Clary sage ***Salvia sclarea*** L.

Melilotus leaves from *Melilotus officinalis* L. have been used to flavour snuff & tobacco. Tonka bean absolute, deertongue absolute & melilotus absolute find some uses in perfumery, but woodruff absolute is no longer much used, apart from flavouring wines. Coumarin derivatives such as the sweetly herbaceous 7-hydroxycoumarin (umbelliferone) also occur in natural products (e.g. in lavender absolute from *Lavandula angustifolia*) but derivatives like herniaren are banned IFRA.



herniaren



umbelliferone

4. *Coumarin in Flavourings.*

Use of coumarin and coumarin-containing herb extracts was common in earlier times. Walter (1916) relates the use of woodruff extract from the fresh flowering herb in the preparation of lemonade, but remarks that it tends to be weak and prone to cause turbidity, and gives an alternative recipe for woodruff flavouring constructed from synthetic coumarin, alcohol and tonka bean tincture. Use of woodruff extract in cola, caramel, gooseberry and other flavourings is also detailed, and the use of tonka essence containing coumarin, vanillin etc is also outlined for flavouring of fondants.

Earlier reports of the toxicity and carcinogenicity of coumarin are now believed to be due to impurities, but coumarin is banned in foods in USA (21CFR 189.130), Japan, India, & the EC, and was banned in Germany from 1970 to 1991 (the ban is now replaced by a concentration limit) etc. Since many derivatives of coumarin are commercial poisons, e.g. warfarin, the well-known rat poison, it has been difficult to persuade people of coumarin's safety. However a detailed discussion of the beneficial uses of *Melilotus* extract & coumarin in phytotherapy (and there are many) & any remaining toxicological issues are given in a *Meliltotus* monograph by Mills and Bone (2000).

The FDA dubiously identified coumarin as a carcinogen in 1954. Subsequent studies initially upheld this opinion, but then disproved it. The net result is that because of the controversy (see elsewhere in this document), it cannot be added to foods (although it is famously naturally present in many, including spices (cinnamon), cherries, apricots, green tea, licorice & strawberries!).

In the EU, flavourings are regulated according to the Articles of the European Council's Directive on food flavourings. Coumarin was placed in Annex II of this Directive (88/388/EEC) in 1988, which was subsequently amended by 91/71/EEC and implemented into UK national law in the Flavourings in Food Regulations 1992: You might remember, that coumarin had been restricted with respect to its allowable concentration in foodstuffs because of allegations of (non-linear dose related) rat & dog carcinogenicity which occurred at high levels of coumarin administration. These considerations caused the regulators to limit coumarin concentrations in food & beverages to 2 mg/Kg, except for limits for chewing gum (50 mg/Kg), alcoholic drinks (10mg/Kg) & caramel confectionery (10mg/Kg). However the EU Scientific Committee for Food (1997) recommended the lowering the coumarin limit to the limit of detection in food, (then 0.5 mg/Kg). We know that the metabolism of coumarin proceeds through a different major route of 7-hydroxylation in humans compared with the 3-hydroxylation pathway in rats (Cohen 1979, Fentem & Fry 1993, Kaighen & Williams 1961, Lake *et al* 1989), further species to species differences being investigated for example by Fenton & Fry (1993), who found that a hepatotoxic route involving 3-hydroxylation and involving a 3,4-epoxide occurs in the rat, but not in baboons, gerbils, some strains of mice, and man. This hypothesis had also been muted by Steensma (1994) amongst others, and was further explored in Lake's paper with Gray (1999), who fed dihydrocoumarin to rats (which cannot form the 3,4 epoxide metabolite) and found no hepatocarcinogenic effect. As was discussed in detail earlier in this document, the existence of genetic polymorphism in humans with regard to the existence of different metabolic detoxification routes for coumarin has brought us to a situation where EFSA in its Opinions in 1994, 1997 & 2004 cannot rule out the possibility of epoxide formation. EFSA considered that the overall NOAEL for liver toxicity in the most sensitive animal species, based on hepatotoxicity in a two year dog study, was 10 mg coumarin/kg bw/day. As mentioned earlier, applying a safety factor of 100, a TDI of 0 - 0.1 mg coumarin/kg bw has been presently established

5. *Coumarin in Perfumery.*

Coumarin has a long history of importance in perfumery, being the first synthetic (synthesised by W.H. Perkin 1868) to be used in a fragrance - *Fougère Royale* (Houbigant) - where coumarin was combined with lavender, citrus and woody notes.- and since that time coumarin has been fundamental to the fougère perfumery accord, together with lavender and bergamot oils. Synthetic coumarin is used in large volumes in fragrances.

Reference:

Bedoukian P. (1993) "Fougère Royale by Houbigant." *Perfumer & Flavorist* **18**, 35 (Sept-Oct 1993)

6. *Coumarin: a Sensitiser (?).*

Only one clinically-relevant case of allergy to coumarin is known to science (Mutterer *et al*. 1999). So why all the wasted toxicological & regulatory effort to restrict the concentration of coumarin in cosmetics?

An article by François Floc'h *et al.* (2002), of Rhodia Perfumery & Specialities, looked at pure coumarin applied in homogenous form to the skin of animals and humans, and concluded that **coumarin is not a dermal allergen**. Coumarin was one of the items you will remember cited in the SCCNFP position paper for Fragrance Allergy in Consumers (SCCNFP/0017/98 final Dec 1999) as being a skin sensitiser, this being the conclusion of previous COLIPA and RIFM opinions. Previous work by Malten K.E. *et al.* (1984), De Groot A.C. *et al*. (1988), Larsen W. *et al.* (1996), and Van Joost T *et al.* (1985) on coumarin was also reviewed by François Floc'h *et al.* who commented, amongst other things, on the lack of scientific rigor, and found no statements of the purity of the materials previously used, and who questioned the homogeneity and the stability of the coumarin in petrolatum suspension. Floc'h *et al.* further indicated the above work failed to distinguish allergy to coumarin and cross-reaction to allergens for which coumarin might be an indicator.

The SCCP Opinion on coumarin as a sensitiser SCCP/0935/05 (adopted 20th June 2006) considers whether coumarin of >99.99% purity had any sensitising properties, & if it doesn't, whether the Opinion on Fragrance Allergy SCCNFP/0017/98 would need to be changed. The committee concluded that coumarin of 99.9% purity when patch tested at 2% would be able to elicit allergic contact reactions in humans. The Opinion is discussed in detail below.

We now return to ground covered above. The paper from Vocansen *et al.* (2007) on the non-allergenicity of pure coumarin, indicates that dihydrocoumarin, an contaminant of impure coumarin, promotes cell proliferation in the LLNA test whereas pure coumarin does not. The authors state that "that pure coumarin is endowed with very weak sensitizing capacities, if any, and suggest that the presence of contaminants in coumarin preparations may account for the previously reported allergenic properties of coumarin

Again covered above, the July edition of the German consumer magazine *Öko-Test*, No. 7/2004, 55, reported on studies done by the IVDK, an information network association of dermatologists, headed up by Prof. Schnuch (Schnuch 2005). It concluded that not all the 26 allergens identified in the SCCNFP Opinion (and subsequently enshrined in the 7th Amendment to the Cosmetics Act) bear the same risk, and criticises the EU Commission for treating them all as equal. Coumarin was identified as This has been followed by a further paper (Schnuch *et al*. 2007) and a lecture (Schnuch 2008) re-iterating the point that the evidence for coumarin being a sensitiser is too weak to consider for regulatory purposes. And that seemed to be that, until IFRA introduced a new set of restrictions for coumarin under its QRA-based 43rd Amendment. If Cropwatch manages to see these documents, which are not available in the public domain, we will report further.

7. Cropwatch Comments on SCCP Opinion SCCP/0935/05 (from July 2006).

Although the SCCP Opinion SCCP/0935/05 heavily criticises various published papers/abstracts/posters by Vocanson *et al.* (2006), Masamoto (2001), CIT (2001) & INSERM (2003/2004) on regarding alleged coumarin sensitisation on various grounds (i.e. is confusing, there is lack of evidence etc.), those very same remarks apply to their own Opinion. The SCCP document is poorly laid out with lack of clear headings, so that it is not immediately apparent which study you are reading about (until the reader has gone over the paper several times). [The key to understanding the Opinion is that new evidence is considered under various headings: Patch testing, Animal data & LLNA (local lymph node assay) studies, with the identity of the study under consideration confusingly set out in normal type face towards the right hand margin at the bottom of the relevant text (instead of as a heading at the top)]. The discussion 3.3.14 needs rewriting with clear references to the work they are criticising.looks – it looks half finished – ans, sloppily, the Vocanson *et al*. paper is not even fully referenced (full details below) .

The SCCP Opinion that coumarin of 99.9% purity when patch tested at 2% would be able to elicit allergic contact reactions in humans, seem to us to be largely based on the findings of the study by Vocanson *et al.* (2006), who claim that they found a reaction of only **one subject in 512 hospital patients** to pure coumarin (although, on a quick read-through, the SCCP Opinion seemingly only accounts for 510). Commercial samples of coumarin with coumarin derivatives as impurities were found to be weak or moderate sensitisers by the Vocanson team. The SCCP seems to have seized on this one reaction and on the reaction of an individual positive from 101 patients positive to the fragrance mix (of which coumarin is not a component) as evidence that coumarin is a sensitiser. Crucially, the discrepancy between the Vocanson team's finding of one positive and the SCCP's reading of two positives is not explained, and presumably the SCCP did not bother to contact the authors for an explanation of why they had dismissed one of these positive reactions.

The SCCP wastes our time reporting on the evaluating an abstract by Masamoto (2001) and concludes, unsurprisingly, there is not enough evidence presented. The SCCP do not provide an explanation of why they were unable to obtain the full paper (maybe they are unable to cope with articles written in Japanese ?).

Cropwatch can only conclude the following:

1. That this SCCP Opinion SCCP/0935/05 only further establishes that the evidence for pure coumarin as a sensitiser is extremely weak. The SCCP defended their previous Opinion on coumarin only by nit-picking at the paper by Vocanson *et al.* (2006).

2. That criticism by the SCCP of the determinations of the purity of coumarin presented in the publications considered is a bit rich, considering Floc'h's remarks (Floc'h 2002) that previous work up by Malten KE *et al.* (1984), De Groot

AC *et al*. (1988), Larsen W. *et al.* (1996), and Van Joost T *et al.* (1985) had not paid any/sufficient attention to the issue. If the SCCNFP themselves had looked at the coumarin purity issue more closely in the first place, they would not have classified coumarin as a sensitiser in SCCNFP/0017/98 final Dec 1999.

3. We feel that the SCCP are now adopting a different set of evaluative criteria towards new submitted evidence on coumarin, in order to make judgments that support their previous Opinions. They are being defensive rather than objective.

4. If the SCCP had contacted the authors of the papers on coumarin sensitisation that they were reviewing in SCCP/0935/05 for clarification/further information, it is probable that a different outcome would have resulted. The fact that this was not done has to be seen as having a political dimension.

5. The matter of whether it should be required by law that coumarin – a weak sensitiser at best - should need labeling as required under the 7th Amendment to the Cosmetics Directive, now needs investigation by Judicial Review. Further, the assumption that natural botanical products which contain coumarin are sensitising also needs reviewing. Deertongue incoloure from *Carphephorus odoratissmus* (J.F. Gemel) syn *Liatris odoratissima* Mich. syn. *Trilisia odoratissima* (J.F. Gmel.) Cass. (which has a high coumarin content) was after all, previously reported non-sensitising by RIFM (Opdyke D.L.J. 1976).

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