Coumarins: Human health tier II assessment

01 July 2016

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl-	91-44-1
2H-1-Benzopyran-2-one	91-64-5
2H-1-Benzopyran-2-one, 6-methyl-	92-48-8
2H-1-Benzopyran-2-one, 3,4-dihydro-	119-84-6
2H-1-Benzopyran-2-one, 7-methyl-	2445-83-2

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS

already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals assessed together in this report are coumarin (CAS No. 91-64-5) and four of its derivatives.

The following synonyms for the chemicals are used in the report to indicate chemical-specific information: coumarin (CAS No. 91-64-5), 3,4-dihydrocoumarin (CAS No. 119-84-6), 4-methyl-7-diethylaminocoumarin (CAS No. 91-44-1), 6-methylcoumarin (CAS No. 92-48-8) and 7-methylcoumarin (CAS No. 2445-83-2).

Import, Manufacture and Use

Australian

The following uses have been identified in Australia (Galleria Chemica).

Coumarin has reported non-industrial uses in medicines (<0.001 %) (TGA); and in certain food additives (FSANZ).

The chemical 4-methyl-7-diethylaminocoumarin has reported non-industrial use in medicines as an excipient in topical preparations (TGA).

International

The following international uses have been identified through: Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; the United States (US) National Library of Medicine's Hazardous Substances Data Bank (HSDB); the US Household Products Database; and various international assessments including from the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP).

All these chemicals have reported cosmetic use as fragrance ingredients, except for 4-methyl-7-diethylaminocoumarin which has reported use as a stabilising agent in cosmetics.

Two of these chemicals have reported domestic uses:

- coumarin as a brightening agent in detergents and as a fragrance ingredient in household products; and
- 4-methyl-7-diethylaminocoumarin in domestic cleaning products.

The SCCP report (2005) stated that 'coumarin is used as an additive in perfumes and fragranced consumer products at concentrations ranging from 0.5% to 6.4% in fine fragrances and at less than 0.01% in detergents' (SCCP, 2005).

Two of these chemicals have reported commercial uses:

- coumarin to neutralise unpleasant odours in rubber, plastics, paints and sprays; and
- 4-methyl-7-diethylaminocoumarin as an optical brightener in paints, optical bleach in textiles, in coatings for paper, as a laser dye, and marking agent.

Two of these chemical have reported site-limited uses:

- coumarin in the electroplating industry; and
- 7-methylcoumarin as an chemical intermediate.

Four of these chemicals have reported non-industrial uses:

- coumarin as a therapeutic anticoagulant and a flavouring agent in the food industry;
- 3,4-dihydrocoumarin and 6-methylcoumarin as flavouring agents in the food industry; and
- 4-methyl-7-diethylaminocoumarin as a therapeutic agent.

Restrictions

Australian

Coumarin for therapeutic use (excluding when present as an excipient) is listed in Schedule 4 of the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP, 2015).

Schedule 4 chemicals are described as 'Substances, the use or supply of which should be by or on the order of persons permitted by State or Territory legislation to prescribe and should be available from a pharmacist on prescription'. Schedule 4 chemicals are labelled with 'Prescription only medicines' or 'Precription animal remedy' (SUSMP, 2015).

No known restrictions have been identified for the other chemicals.

International

Coumarin and 6-methylcoumarin are listed on the following (Galleria Chemica):

- European Union (EU) Cosmetics Regulation 1223/2009 Annex III—List of substances prohibited in cosmetic products except subject to the restrictions laid down; and
- New Zealand Cosmetic Products Group Standard—Schedule 5: Components cosmetic products must not contain except subject to the restrictions and conditions laid down.

In cosmetics, the presence of coumarin must be indicated in the list of ingredients when its concentration exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products (CosIng; Galleria Chemica).

Coumarin has been prohibited as a food additive in the United States and in the EU, but authorised as a natural component of certain food additives in the EU (Galleria Chemica).

In oral cosmetic products, 6-methylcoumarin is restricted to a maximum concentration of 0.003 % in ready for use preparations (CosIng).

3,4-Dihydrocoumarin when used as a fragrance ingredient, and 7-methylcoumarin are listed on the following (Galleria Chemica):

- Association of South East Asian Nations (ASEAN) Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products;
- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products; and
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain.

No known restrictions have been identified for 4-methyl-7-diethylaminocoumarin.

Existing Worker Health and Safety Controls

Hazard Classification

These chemicals are not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available for any of these chemicals.

International

No specific exposure standards are available for any of these chemicals.

Health Hazard Information

Toxicokinetics

In humans, coumarin is rapidly absorbed from the gastrointestinal (GI) tract after oral administration and is subjected to first pass metabolism by the liver. Only 2–6 % of the absorbed chemical reaches the systemic circulation. Coumarin has also been shown to be easily absorbed via human skin (60 % absorption in humans exposed to a 2 mg dermal dose for six hours) (IARC,

2000). The half-life of coumarin in the human body was reported to be 0.8 hours following oral exposure and 1.02 hours following intravenous (i.v.) administration (VKM, 2010).

In rats, coumarin is rapidly absorbed following oral administration and extensively metabolised, with little excretion of unchanged coumarin. There was no significant tissue accumulation of coumarin or coumarin metabolites in rats following oral exposure (IARC, 2000). Due to enterohepatic circulation in rats, the half-life of coumarin is longer in rats compared with humans (VKM, 2010).

In humans, coumarin is metabolised primarily into 7-hydroxycoumarin in the liver by cytochrome P450 2A6 (CYP2A6) and excreted in the urine as glucuronic acid and sulfate conjugates (IARC, 2000). The other metabolic pathways include transformation into ortho-hydroxyphenylacetic acid (o-HPAA). The chemical 7-hydroxycoumarin represented 79 % of the excreted dose, while 4 % was o-HPAA, when human subjects received oral doses of coumarin at 200 mg/subject (IARC, 2000).

In rats, the major metabolic pathway of coumarin is through 3,4-epoxidation, and excretion in faeces. The 7-hydroxylation pathway is minor, with 7-hydroxycoumarin accounting for less than 1 % of the excreted dose in rat studies (IARC, 2000). In rats, coumarin can also be metabolised to 3,4-dihydrocoumarin by the gastrointestinal microflora under anaerobic conditions (IARC, 2000). As 3,4-dihydrocoumarin lacks the 3,4-double bond, it is considered unlikely to transform into an epoxy intermediate (NTP, 1993a).

Conversion of epoxy intermediates into ortho-hydroxyphenylacetaldehyde (o-HPA) is rapid, but rats have a slower clearance of this toxic metabolite compared with humans and mice (20-50 times slower oxidation rate of o-HPA to o-HPAA in rats compared with humans and mice), which explains the high sensitivity of rats to coumarin with respect to liver toxicity (VKM, 2010).

Acute Toxicity

Oral

These chemicals have different degrees of acute oral toxicity. Based on the available median lethal dose (LD50) values in animals, 7-methylcoumarin and 4-methyl-7-diethylaminocoumarin have low acute oral toxicity. The other three chemicals have moderate to high acute oral toxicity warranting hazard classification.

The following LD50 values were reported.

- for coumarin: 290–680 mg/kg bw in various rat strains and 196–780 mg/kg bw in various mouse strains (VKM, 2010);
- for 3,4-dihydrocoumarin: 1460 mg/kg bw in rats and 1760 mg/kg bw in guinea pigs (NTP, 1993b);
- for 6-methylcoumarin: 1680 mg/kg bw in rats (HSDB);
- for 7-methylcoumarin: 3800 mg/kg bw in rats (ChemIDPlus); and
- for 4-methyl-7-diethylaminocoumarin: 5000 mg/kg bw in rats and 1780 mg/kg bw in mice (ChemIDPlus).

Single doses of coumarin at 120–500 mg/kg bw caused acute hepatotoxicity in Sprague Dawley (SD) and Wistar rats (IARC, 2000; VKM, 2010). A single dose of 200 mg/kg bw of coumarin to mice caused liver necrosis and increased plasma aminotransferase activity (VKM, 2010). Twenty four hours after oral gavage administration of coumarin at 150 mg/kg bw or greater, pulmonary damage was observed in B6C3F1 mice with selective injury to the Clara cells in the terminal bronchioles of the lungs (IARC, 2000; VKM, 2010).

Dermal

Based on the available data for two of the chemicals, these chemicals are expected to have low acute dermal toxicity.

The following dermal LD50 values were reported:

- >5000 mg/kg bw in rabbits for 3,4-dihydrocoumarin (SCCNFP, 2000);

- >5000 mg/kg bw in rabbits for 6-methylcoumarin (HSDB).

No data are available for the other three chemicals.

Inhalation

No data are available for any of the chemicals.

Corrosion / Irritation

Skin Irritation

Two of the chemicals are reported to be mild and moderate skin irritants. However, the available data are insufficient to derive a conclusion on the skin irritation potential of these chemicals.

Application of 3,4-dihydrocoumarin and 6-methylcoumarin on intact or abraded rabbit skin for 24 hours under occlusive conditions was reported to cause moderate and mild irritation, respectively (Opdyke, 1979). No irritation scores were available.

No data are available for the other three chemicals.

Eye Irritation

No data are available for the chemicals.

Observation in humans

Application of 3,4-dihydrocoumarin at 20 % in petrolatum produced no skin irritation after a 48-hour closed patch test in 25 human subjects (SCCNFP, 2000).

Sensitisation

Skin Sensitisation

Out of these five chemicals, four had data on skin sensitisation, the exception being 4-methyl-7-diethylaminocoumarin. Only 3,4-dihydrocoumarin is identified as a skin sensitiser (see also **Sensitisation - Observation in Humans**), warranting hazard classification.

The available data on pure coumarin indicate no skin sensitisation potential. However, some coumarins with impurities that are skin sensitisers, have shown positive results in skin sensitisation studies. The available data indicate that 6-methylcoumarin may have some photoallergenic potential (see also **Sensitisation - Observation in Humans**). 7-Methylcoumarin at 20 % showed no skin sensitising potential.

Skin sensitisation data are available for coumarin, 3,4-dihydrocoumarin, 6-methylcoumarin and 7-methylcoumarin, although some studies are of limited value due to the lack of detail.

In a local lymph node assay (LLNA) following OECD Test Guideline (TG) 429, three coumarin preparations, pure coumarin (purity >99 %), coumarins A and B produced by other methods and of unknown purity, were tested on female CBA/j and BALB/c mice (n = 4/group) at doses of 10, 25 and 50 % in N,N-dimethylformamide (DMF). Coumarins A and B contained the impurities 6-chlorocoumarin, benzochromene and 3,4-dihydrocoumarin. These impurities were also tested at 2.5, 5 and 10 %

concentrations (in DMF) in the LLNA. Pure coumarin did not induce significant cell proliferation, indicating no skin sensitising potential. A stimulation index (SI) of 3.2 was derived for coumarin A in 2/4 animals at the highest concentration and SI values of 3.5 and 3.7 were reported for coumarin B in 1/4 animals at 25 % and 50 %, respectively. All impurities had SI >3 at the highest concentration (10 %) in at least half of the animals, indicating that these impurities are skin sensitisers (SCCP, 2005). Another LLNA conducted using EEC 96/54/EC Part B, Method B.6 guidelines reported coumarine (Rhodiascent TM Coumarine - purity not available) as not sensitising based on SI values of less than three derived for 5%, 10% and 25% concentrations in acetone/olive oil (4/1, v/v) (SCCP, 2005).

In a combined study which used female mice in a mouse ear swelling test (MEST) and a LLNA, pure coumarin was found negative while 3,4-dihydrocoumarin proved to be a contact sensitiser. In the MEST, mice were treated on three consecutive days by epicutaneous application of 3,4-dihydrocoumarin or pure coumarin at 50 % onto abdominal skin, and challenged five days later with a non-irritant concentration of 3,4-dihydrocoumarin or pure coumarin at 40 % onto the left ear. In the LLNA, mice were administered the chemical (topical, 25 mL/ear) on both ears for three consecutive days (Vocansson, 2007).

Two guinea pig studies for photo-sensitisation are available for 6-methylcoumarin. In one study, guinea pigs (n = 10/sex) were initially induced epicutaneously, four times a week with a 0.1 % w/w solution of 6-methylcoumarin. The animals were induced again with four intradermal injections of Freund's complete adjuvant (FCA)/saline mixture (1:1) during the second and third weeks. The animals were challenged epicutaneously for three consecutive days on weeks six and nine with a 0.1 % w/w solution of 6-methylcoumarin. Animals were irradiated after each challenge application. No evidence of photoallergenic potential was observed in guinea pigs at the dose tested (Maurer et al., 1980).

In the second study, ten Hartley guinea pigs were induced with 6-methylcoumarin using two methods : 1) cutaneous exposure followed by irradiation, five times within two weeks, and 2) first cutaneous exposure combined with intradermal injection of FCA, then five-time exposure followed by irradiation, within two weeks. In the challenge phase, guinea pigs were exposed topically to 6-methylcoumarin at 10, 1 and 0.1 % and observed 24 or 48 hours later. No photoallergic contact dermatitis was detected in animals induced topically. However, the animals induced by intradermal injection showed a positive reaction for photosensitisation in more than 85 % of the animals at 1 % and 10 % challenge concentrations. At 1 % challenge concentration, 45 % of the animals were sensitised, but no skin reactions were observed at 0.1 % (Ichikawa et al., 1981).

In a FCA test, 7-methylcoumarin at 20 % did not induce any skin sensitisation reaction in guinea pigs (n = 10) (Hausen & Kallweit, 1986).

Observation in humans

In a clinical study, coumarin was tested in 379 patients; 100 were patch tested with coumarin at 1 % and 10 %, while the remaining 279 were patch tested with coumarin at 2 %. Except for one patient reacting to several chemicals including coumarin at 2 %, no reactions were observed (SCCP, 2005). In another clinical study, when 101 patients with allergic skin reactions to a fragrance mix was tested, only one patient was positive to coumarin at 2 % (SCCP, 2005). Another study with 30 patients who had positive patch test reactions to their own perfume showed no positive skin reactions to coumarin at 2% (SCCP, 2005).

Application of 3,4-dihydrocoumarin at 20% in petrolatum produced skin sensitisation reactions in all 25 volunteered test subjects (SCCNFP, 2000).

In a photomaximisation test, 6-methylcoumarin was applied in 25 subjects for 24 hours followed by exposure to three minimal erythema doses (MED) of solar simulated radiation twice weekly for three weeks (six exposures). The MEDs were individually determined by administering a series of doses in which each dose was 25 % greater than the previous one. The subjects were then challenged two weeks later, with an occlusive patch for 24 hours followed by solar radiation. Results showed photocontact allergy in a high proportion of test subjects: 13 became photocontact sensitive, two developed both contact and photocontact sensitivity and two had plain contact allergy (Kaidbey et al., 1980).

In a maximisation test in 25 volunteers, 6-methylcoumarin at 4 % in petrolatum produced no skin reactions (HSDB).

Repeated Dose Toxicity

Oral

Based on the available data for coumarin and 3,4-dihydrocoumarin, these five chemicals are not considered to cause severe systemic health effects following repeated oral exposure.

Repeated dose, 13-week studies in rats and mice were conducted by the NTP on coumarin (NTP, 1993a) and 3,4-dihydrocoumarin (NTP, 1993b). Coumarin caused liver toxicity in rats at high doses (>150 mg/kg bw/day), and to a lesser extent in mice. In contrast, no treatment-related liver lesions were observed in rats that received 3,4-dihydrocoumarin at 600 mg/kg bw/day. Results from 13-week gavage studies on rats are presented below.

Coumarin in corn oil was administered to Fischer 344/N (F344/N) rats (n = 10/sex/dose) at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw/day, five days per week for 13 weeks. Three females and three males died before the end of the study at 300 mg/kg bw/day. Rats receiving the chemical at 150 and 300 mg/kg bw/day had centrilobular hepatitis, consisting in a spectrum of changes including centrilobular hepatocyte degeneration and necrosis, chronic active inflammation and bile duct hyperplasia. The absolute and relative liver weights were significantly increased at 150 or 300 mg/kg bw/day, compared with controls; and the absolute and relative thymus weight was significantly decreased at 300 mg/kg bw/day, compared with controls, presumably in response to the stress associated with liver toxicity. Final mean body weight was significantly decreased in males at 150 and 300 mg/kg bw/day, compared with the control group. At 75 mg/kg bw/day and above, there was a statistically significant dose-related decrease in the mean erythrocyte volume and mean erythrocyte haemoglobin level, and dose-related increases in the erythrocyte count. However, these changes in blood parameters were not considered clinically significant, compared with controls (NTP, 1993a). A no observed adverse effect level (NOAEL) was not identified.

The chemical 3,4-dihydrocoumarin (in corn oil) was administered to F344/N rats (n = 10/sex/dose) at doses of 0, 75, 150, 300, 600, or 1200 mg/kg bw/day, five days per week for 13 weeks. Two males and five females died at the highest dose. Absolute and relative liver and kidney weights were significantly increased in males and females at 600 and 1200 mg/kg bw/day, with hepatocellular hypertrophy at 300, 600 and 1200 mg/kg bw/day. Platelet counts were significantly decreased in both sexes at 600 and 1200 mg/kg bw/day and also in females at 300 mg/kg bw/day. Haemoglobin and haematocrit values and erythrocyte counts were significantly decreased in males at 300 mg/kg bw/day and above (NTP, 1993b). No NOAEL was identified.

In a 13-week comparative study, SD rats were fed with equimolar doses of 0.75 % coumarin, 0.76 % 3,4-dihydrocoumarin or 0.82 % 6-methylcoumarin (5.14 mmol/100 g diet). All treated animals showed increased relative liver weights and activated hepatic GSHS-transferase and gamma-glutamyltransferase enzymes (activation was high in rats fed with coumarin and 6-methylcoumarin). Only coumarin increased plasma alanine aminotransferase and aspartate aminotransferase activities, and induced vacuolation of centrilobular hepatocytes and bile duct hyperplasia. Cholangiofibrosis was also observed, particularly in rats given 0.75 % coumarin. The chemical 3,4-dihydrocoumarin produced no effects, whereas 6-methylcoumarin induced a slight vacuolation of individual hepatocytes in some rats (Lake et al., 1994).

In a two-year feeding study, groups of rats (n = 5/sex/dose) were given 6-methylcoumarin at 0, 500, 1000, 3500, 7500 or 15000 ppm (approximately equivalent to 0, 25, 50, 175, 375 and 750 mg/kg bw/day). Treatment-related effects were observed only at high doses and included growth depression (in males at 7500 ppm and in both sexes at 15000 ppm), and microscopic liver changes and moderate atrophy at the highest dose (Opdyke, 1979).

In a poorly described study, a dog fed with 6-methylcoumarin at 150 mg/kg bw (frequency not stated) showed signs of emaciation, dehydration and weakness after 39 days, with moderate to severe hepatitis and slight to moderate muscle atrophy (Opdyke, 1979). However, another study reported no toxicity effects in one male and one female dog which received 6-methylcoumarin at 50 mg/kg bw/day for two years (Opdyke, 1979).

In a two-year feeding study in dogs, coumarin caused histological lesions in the liver at the dose of 25 mg/kg bw/day within 100 days but no effects were reported at 10 mg/kg bw/day, which was determined as the NOAEL (EFSA, 2004).

No data were available on 7-methylcoumarin and 4-methyl-7-diethylaminocoumarin.

Dermal

No data are available for the chemicals.

Inhalation

No data are available for the chemicals.

Observation in humans

Reports from clinical trials indicate that coumarin does not cause severe health effects following repeated oral exposure, but some liver effects have been observed in a small proportion of patients (IARC, 2000). Two cases of hepatotoxicity were reported from clinical trials on 1106 lymphoedema patients taking 400 mg/day of coumarin for a mean duration of 14.6 months (IARC, 2000). In another clinical trial on 2173 patients treated with coumarin (at 100 mg/day for a month, and then 50 mg/day for two years), eight of them (0.37 %) developed elevated serum aminotransferase levels (IARC, 2000).

A tolerable daily intake (TDI) of 0.1 mg/kg bw/day for coumarin was determined for human exposure, based on a NOAEL of 10 mg/kg bw/day from a two-year oral study in dogs (EFSA, 2004).

Genotoxicity

Based on the overall information, including the negative in vivo genotoxicity data reported for coumarin, the chemicals in this report are not expected to be genotoxic.

Mixed results were reported for in vitro genotoxicity studies with coumarin, without further details of the studies:

- negative results in bacterial gene mutation tests using *Salmonella typhimurium* strains TA98, TA1535, TA1537 and TA1538, with or without metabolic activation (VKM, 2010);
- positive for gene mutations in *S. typhimurium* strain TA100, only with metabolic activation at 2000 µg/plate (VKM, 2010);
- sister chromatid exchanges (SCE) were induced in Chinese hamster ovary (CHO) cells, only without metabolic activation at 50–500 µg/mL (VKM, 2010);
- a dose-dependent increase in chromosomal aberrations in another SCE assay in CHO cells, only with metabolic activation at 160–1600 µg/mL, but negative results without metabolic activation (VKM, 2010);
- no chromosome aberrations in CHO cells at doses up to 333 µM (VKM, 2010);
- no gene mutation at the *hprt* locus of CHO cells (IARC, 2000);
- positive results in a micronucleus assay in human hepatoma cells but not in rat primary hepatocytes (IARC, 2000); and
- no unscheduled DNA synthesis (UDS) in human hepatocytes up to 5 mM (VKM, 2010).

A limited number of in vitro genotoxicity studies were available for 3,4-dihydrocoumarin, 6-methylcoumarin and 4-methyl-7-diethylaminocoumarin, indicating mixed results without further details of the studies:

- 3,4-dihydrocoumarin gave negative results in bacterial gene mutation tests using *S. typhimurium* strains TA100, TA1535, TA1537 and TA98, with or without metabolic activation, at doses up to 6666 µg/plate (CCRIS; NTP, 1993b); induced SCE in a dose-related manner in CHO cells from 50 to 300 µg/mL, without metabolic activation, and a significant increase in SCE at 1600 and 2000 µg/mL with metabolic activation (NTP, 1993b);
- 6-methylcoumarin gave only a weak response in an Ames test, but induced chromosome aberrations in CHO cells (HSDB); and
- 4-methyl-7-diethylaminocoumarin was negative in bacterial gene mutation tests which used *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, with or without metabolic activation, up to 5450 ug/plate (CCRIS).

The following results were obtained from in vivo assays:

- in a micronucleus assay, oral administration of coumarin at 50, 100 or 200 mg/kg bw to mice did not increase the incidence of micronucleated polychromatic erythrocytes (EFSA, 2004);
- Groups of SD rats and F344/N rats exposed orally to coumarin at doses of 60, 120 or 240 mg/kg bw and 25, 50 or 100 mg/kg bw, respectively, showed no DNA covalent binding (adduct formation) in the liver or kidneys (EFSA, 2004);

- in an unscheduled DNA synthesis (UDS) assay in male SD rats which received coumarin at doses of 32, 107 or 320 mg/kg bw, there were no UDS in rat hepatocytes (EFSA, 2004);
- coumarin did not induce any sex-linked recessive lethal mutations in *Drosophila melanogaster* (details not available) (IARC, 2000);
- 3,4-dihydrocoumarin did not induce micronucleated normochromatic erythrocytes in mice exposed to the chemical for 13 weeks at doses up to 1600 mg/kg bw/day (NTP, 1993b); and
- 6-methylcoumarin did not induced chromosome aberrations when injected to mice (dose not available) (HSDB).

Coumarin has been used to modulate the mutagenic activity of other chemicals, therefore 'has been generally considered as an antimutagen', 'however, (…) it also acts as a co-mutagen in some assays' (IARC, 2000).

Carcinogenicity

Rodent studies with coumarin and 3,4-dihydrocoumarin showed some evidence of carcinogenic activity, but were not sufficiently conclusive to classify the chemicals as carcinogenic.

The IARC has classified coumarin as ‘Not classifiable as to its carcinogenicity to humans' (Group 3), based on limited evidence for carcinogenicity in animal tests.

In vivo genotoxicity data for coumarin were reported to support a 'non-genotoxic mode of action for tumour induction' (EFSA, 2004).

Carcinogenicity studies on rats and mice were undertaken by the NTP on coumarin (NTP, 1993a) and 3,4-dihydrocoumarin (NTP, 1993b). Results from these 103-week gavage studies are presented below.

Groups of F344/N rats (n = 50/sex/dose) received coumarin in corn oil by gavage at doses of 0, 25, 50 or 100 mg/kg bw/day. Low numbers of renal tubule adenomas were observed in male rats of all groups (1/50, 2/49, 2/51, 1/50) and in females (2/49) at 100 mg/kg bw/day. The incidence of this uncommon neoplasm was reported as not significantly greater than those of the controls but higher than the incidence in NTP historical controls. There was a dose-related increase in the severity of bile duct hyperplasia, but significant only in males at 100 mg/kg bw/day. No chemically-related increased incidences of liver neoplasms were observed (NTP, 1993a).

Groups of B6C3F1 mice (n = 50/sex/dose) received coumarin in corn oil by gavage at doses of 0, 50, 100 or 200 mg/kg bw/day. The study showed 'some evidence of carcinogenic activity' in male mice and 'clear evidence of carcinogenic activity' in female mice. There was a significantly increased incidence of alveolar bronchiolar adenomas at 200 mg/kg bw/day (24/51 in male and 20/51 in female mice) compared with the controls (14/50 and 2/51, in males and females, respectively). In addition, the high-dosed females exhibited a significant increase of the incidence of alveolar bronchiolar carcinomas (7/51) compared with the control (0/51), but not the high-dosed males (1/51) compared with the control (1/50). A statistically significant increase in the incidence of hepatocellular adenomas was observed in the 50 and 100 mg/kg bw/day female mice, but not at 200 mg/kg bw/day (8/50, 26/49, 29/51, 12/50 at 0, 50, 100 and 200 mg/kg bw/day respectively). In male mice, the incidence was similar to that of the controls (26/50, 29/50, 29/50, 27/51 at 0, 50, 100 and 200 mg/kg bw/day respectively) (NTP, 1993a).

Groups of F344/N rats (n = 60/sex/dose) received 3,4-dihydrocoumarin in corn oil by gavage at doses of 0, 150, 300 or 600 mg/kg bw/day. In male rats, there were increased incidences of renal focal hyperplasia (0/50, 5/48, 6/47, 8/50) and tubule adenomas (1/50, 1/48, 3/47, 6/50), exceeding that of historical controls. Two male rats also exhibited transitional cell carcinomas in the kidneys at the highest dose. There was no evidence of carcinogenic activity in females at 150, 300, or 600 mg/kg bw/day (NTP, 1993b).

In B6C3F1 mice (n = 70/sex/dose) administered 3,4-dihydrocoumarin at 0, 200, 400 or 800 mg/kg bw/day for 103 weeks, the chemical showed no evidence of carcinogenic activity in males up to the highest dose. There was some evidence of carcinogenic activity in females based on significantly increased incidences of hepatocellular adenoma (10/51, 20/50, 22/50, 20/52), and hepatocellular adenoma or carcinoma (combined) (13/51, 21/50, 25/50, 24/52), significant only at 400 and 800 mg/kg bw/day (NTP, 1993b).

No carcinogenicity data are available for the other three chemicals.

Reproductive and Developmental Toxicity

Based on the limited information available, no conclusion can be derived on the reproductive and developmental toxicity of these chemicals.

One reproductive study is available on coumarin and no data are available for the other chemicals.

Groups of 26–30 female NMRI mice were orally exposed to coumarin in the diet at 0, 0.05, 0.1 and 0.25 % on gestation days (GD) 6–17. There was no effect on the total number of implantations, resorptions or foetal deaths, and no reduction in foetal weight (IARC, 2000). There were no malformations observed in foetuses, but there was an increased numbers of stillbirths and delayed ossification at the highest dose. Increased mortality in pups up to three weeks of age was observed at all dose levels (NTP, 1993a).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include acute oral toxicity for coumarin, 3,4-dihydrocoumarin and 6-methylcoumarin. The chemical 3,4-dihydrocoumarin is also a skin sensitiser. The chemical 6-methylcoumarin, a synthetic fragrance ingredient, may have some potential for photo-sensitisation effects.

Based on the available data, critical health effects are not identified for two of the chemicals (7-methylcoumarin and 4-methyl-7-diethylaminocoumarin), although they can be considered likely to have one or more of the same critical effects of the other chemicals assessed in this report.

No data were available on acute inhalation toxicity, skin and eye irritation, repeated dose dermal and inhalation toxicity for any of these chemicals. Only limited data are available on reproductive and developmental toxicity of coumarin, but not on the other derivatives in this report.

Public Risk Characterisation

Although no cosmetic or domestic uses in Australia are identified, these chemicals have reported cosmetic and domestic uses overseas, mainly as fragrance ingredients in cosmetic and consumer products, including domestic cleaning products.

Coumarin is listed on Schedule 4 of the SUSMP for therapeutic use (excluding when used as an excipient). There are no restrictions in Australia on using these chemical in cosmetic or domestic products, although there are restrictions overseas (see **Restrictions - International**).

In the absence of regulatory controls, the characterised critical health effects (skin sensitisation) for 3,4-dihydrocoumarin have the potential to pose an unreasonable risk if used in cosmetic or domestic products, although the available data are insufficient to determine the sensitisation potency.

Coumarin, 6-methylcoumarin and 7-methylcoumarin are not expected to pose an unreasonable risk to public health if used in cosmetic or domestic products at low concentrations (see **Restrictions - International)**.

As the sensitisation potential has not been identified for 4-methyl-7-diethylaminocoumarin, should this chemical be used in cosmetic and/or domestic products, there could be a potential for an unreasonable risk to public health.

Overall, there is uncertainty regarding the safety of 3,4-dihydrocoumarin and 4-methyl-7-diethylcoumarin in cosmetic and domestic products; therefore, a Tier III assessment may be required, depending on the outcomes of industry consultations (see **Recommendation**), to determine the extent of use in Australia and the availability of toxicity data.

Occupational Risk Characterisation

During product formulation, oral and dermal exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical health effects identified for coumarin, 3,4-dihydrocoumarin and 6-methylcoumarin, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal exposure are implemented. These three chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support a recommendation for hazard classification of coumarin, 3,4-dihydrocoumarin and 6-methylcoumarin (refer to **Recommendation** section).

Overall, there is uncertainty regarding the hazards of 4-methyl-7-diethylcoumarin in the workplace; therefore, a Tier III assessment may be required, depending on the outcomes of industry consultations (see **Recommendation**), to determine the extent of use in Australia and the availability of other toxicity data.

NICNAS Recommendation

Further risk management is required. Sufficient information is available to recommend that risks for workplace health and safety be managed for coumarin, 3,4-dihydrocoumarin and 6-methylcoumarin through changes to classification and labelling.

It is also recommended that NICNAS undertake further consultation with industry to determine to what extent 3,4-dihydrocoumarin and 4-methyl-7-diethylaminocoumarin are used in Australia, and the specialised containment measures that may be required based on the pattern or type of use.

Should the consultation identify additional evidence pertaining to the use of the chemicals that may pose a risk to workers and/or the public, a Tier III assessment should be undertaken to characterise the risk and recommendations required if considered appropriate.

Regulatory Control

Public Health

Products containing coumarin should be labelled in accordance with state and territory legislation (SUSMP, 2015).

Work Health and Safety

The following chemicals are recommended for classification and labelling under the current approved criteria and adopted GHS as below. The other chemicals do not require a hazard classification.

Coumarin:

Xn; R22 (Acute Toxicity Cat.3 H301)

6-methylcoumarin:

Xn; R22 (Acute Toxicity Cat.4 H302)

The table below provides the hazard classification recommended for 3,4-dihydrocoumarin.

This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)	Harmful if swallowed - Cat. 4 (H302)
Sensitisation	May cause sensitisation by skin contact (Xi; R43)	May cause an allergic skin reaction - Cat. 1 (H317)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing these chemicals should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral or dermal exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using local exhaust ventilation to prevent the chemicals from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;

- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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Chemical Identities

Chemical Name in the Inventory and Synonyms	**2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl-** 4-methyl-7-diethylaminocoumarin 7-diethylamino-4-methylcoumarin 7-(diethylamino)-4-methyl-2H-1-benzopyran-2-one coumarin, 7-(diethylamino)-4-methyl-
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	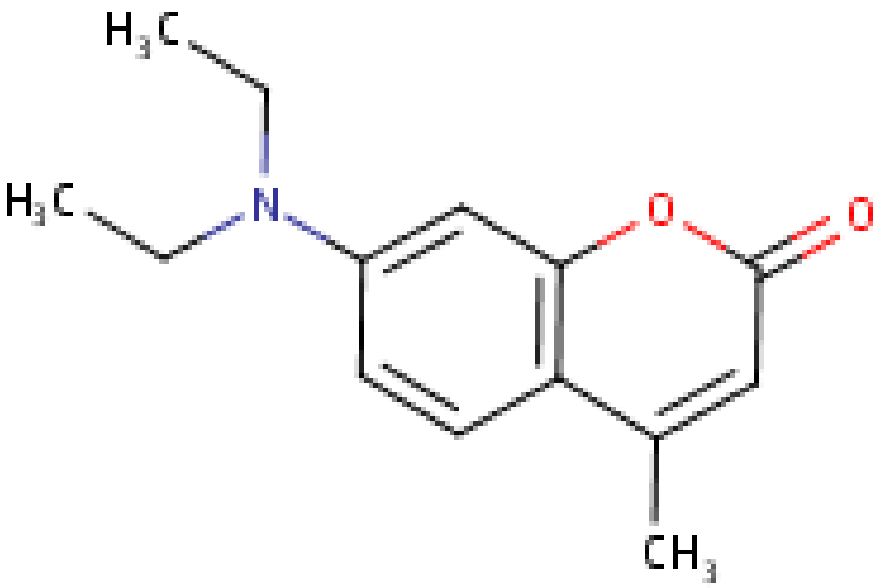
Molecular Formula	C14H17NO2
Molecular Weight	231.3

Chemical Name in the Inventory and Synonyms	**2H-1-Benzopyran-2-one** coumarin 1,2-benzopyrone 2-oxo-1,2-benzopyran 2-propenoic acid, 3-(2-hydroxyphenyl)-, delta-lactone 5,6-benzo-alpha-pyrone
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	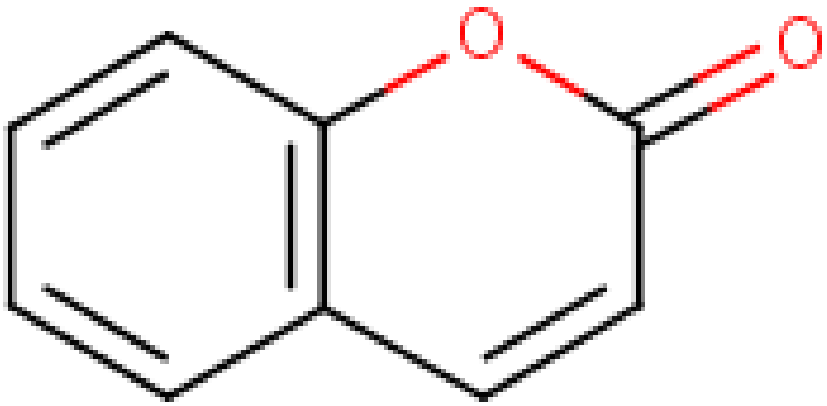
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Chemical Name in the Inventory and Synonyms	**2H-1-Benzopyran-2-one, 6-methyl-** 6-methylcoumarin 5-methyl-2-hydroxyphenylpropenoic acid lactone 6-methylbenzopyrone pralina toncarine
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	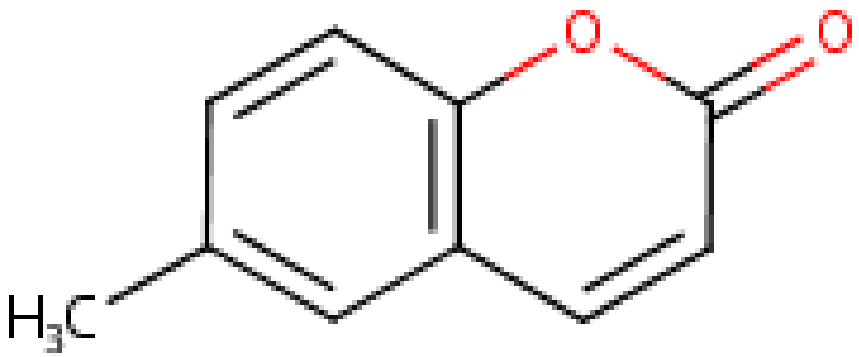
Molecular Formula	C10H8O2
Molecular Weight	160.1712

Chemical Name in the Inventory and Synonyms	**2H-1-Benzopyran-2-one, 3,4-dihydro-** 3,4-dihydrocoumarin 1,2-benzodihydropyrone 2-chromanone melilotin 2-hydroxydihydrocinnamic acid lactone
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	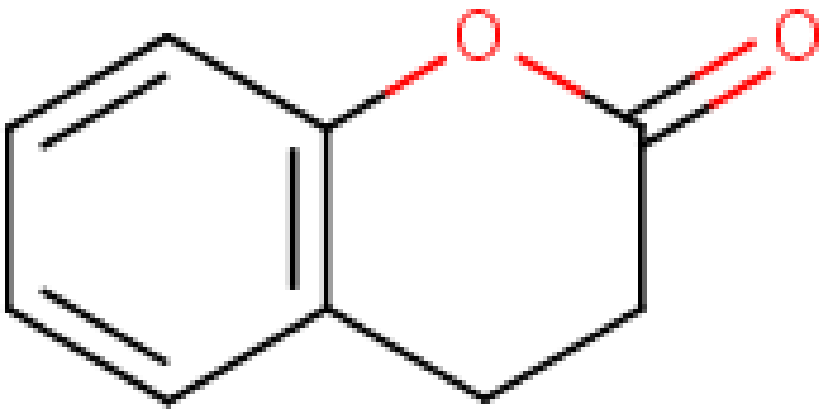
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	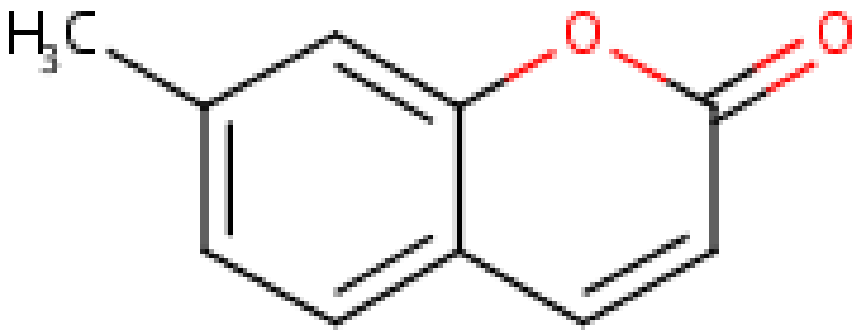
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Molecular Weight	160.171

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