COUMARIN

This substance was considered by previous Working Groups, in October 1975 (IARC, 1976) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

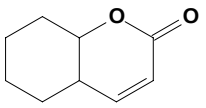
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 91-64-5
Chem. Abstr. Name: 2*H*-1-Benzopyran-2-one
IUPAC Systematic Name: Coumarin
Synonyms: 1,2-Benzopyrone; 5,6-benzo-2-pyrone; benzo-α-pyrone; *cis-ortho*-coumarinic acid lactone; coumarinic anhydride; *ortho*-hydroxycinnamic acid lactone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_6O_2$ Relative molecular mass: 146.15

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Orthorhombic, rectangular plates; pleasant, fragrant odour resembling that of vanilla beans; burning taste (Budavari, 1998)
(*b*) *Boiling-point*: 301.7 °C (Lide, 1999)
(*c*) *Melting-point*: 71 °C (Lide, 1999)
(*d*) *Density*: 0.935 g/cm^3 at 20 °C (Lide, 1999)

(*e*) *Spectroscopy*: Infrared (prism [1691]; grating [270]), ultraviolet [492], nuclear magnetic resonance (proton, [10407, V-225]; C-13 [242]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (100 mg/L at 25 °C) and ethanol; very soluble in chloroform, diethyl ether and pyridine (Lide & Milne, 1996; Verschueren, 1996; Budavari, 1998)
(*g*) *Volatility*: Vapour pressure, 0.13 kPa at 106 °C (Verschueren, 1996)
(*h*) *Octanol/water partition coefficient (P)*: log P, 1.39 (Verschueren, 1996).
(*i*) *Conversion factor*[1]: $mg/m^3 = 5.98 \times ppm$

1.1.4 *Technical products and impurities*

Coumarin is commercially available with a minimum purity of 99% (Rhodia, undated). Coumarin is usually sold in the form of colourless shiny leaflets or rhombic crystals (Boisde & Meuly, 1993).

1.1.5 *Analysis*

Coumarin can be determined in vanilla extract by a photometric method, reading the absorbance or transmittance at 490 nm, and comparing against a standard (AOAC International, 1998).

1.2 Production

Until the late 1890s, coumarin was obtained commercially only from natural sources by extraction from tonka beans. Synthetic methods of preparation and industrial manufacturing processes were developed starting principally from *ortho*-cresol (Raschig process), phenol (Pechmann reaction) and salicylaldehyde (Perkin reaction). Various methods can be used to obtain coumarin from each of these starting materials. In order to be suitable for perfumery uses, synthetic coumarin must be highly pure (Bauer *et al*., 1988; Boisde & Meuly, 1993).

Information available in 1999 indicated that coumarin was manufactured by five companies in China, three companies each in Japan and the United States, two companies in France and one company each in Germany, Hong Kong and India (Chemical Information Services, 1999).

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[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming a temperature of 25 °C and a pressure of 101 kPa

1.3 Use

Coumarin is widely distributed in the plant kingdom, but for commercial use has been mostly produced synthetically for many years. In addition to its use in the perfumery, cosmetic and related industries, coumarin has several other industrial applications. Formerly, large quantities of coumarin were used in the food industry, mostly associated with vanillin, for flavouring chocolates, baked goods, and in cream soda-flavoured beverages (Perone, 1972), but since 1954 its use as a direct food additive has been suspended in the United States (Boisde & Meuly, 1993; Lake, 1999).

Because of its unique sweet note and stability, coumarin has long been recognized as an important raw material in the fragrance industry. It is widely used in hand soaps, detergents, lotions and perfumes at concentrations usually extending from 0.01 to 0.8%. It is normally associated in perfumery with herbaceous odours and enters into the formulation of fern and Chypre-type fragrances. It is used as an odour-enhancer to achieve a long-lasting effect when combined with natural essential oils such as lavender, citrus, rosemary and oak moss. Coumarin is used in tobacco to enhance its natural aroma. It is also applied in large quantities to give pleasant aromas to household materials and industrial products or to mask unpleasant odours (Egan *et al*., 1990; Boisde & Meuly, 1993).

Coumarin has also found use in toothpastes, antiperspirant deodorants, bath products, body lotions, face creams, fragrance creams, hair sprays, shampoos, shower gels and toilet soaps (Cohen, 1979; Lake, 1999). It has been used in detergents as a brightener or bleaching agent (Perone, 1972).

Coumarin possesses both immunomodulatory and direct antitumour activity (Marshall *et al.*, 1994).

Coumarin has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. It is currently undergoing clinical trials for treatment of lymphoedema following breast cancer treatment and in treatment of lung and kidney cancer and of melanoma alone or in combination with cimetidine (Marshall *et al*., 1987a,b, 1989; Thornes *et al*., 1989; Dexeus *et al*., 1990; Marshall *et al*., 1990; Casley-Smith *et al*., 1993; Lake, 1999). It has also been used for prevention of dental caries (Perone, 1972). Coumarin and some of its derivatives have been tested for treatment of schizophrenia, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals (Boisde & Meuly, 1993).

In industry, it is used in rubber and plastic materials and in paints and sprays to neutralize unpleasant odours (Lake, 1999). In other fields, coumarin has a significant use in the electroplating industry, mostly in the automotive area, to provide high polished quality to chrome-plated steel, but this use is declining (Egan *et al*., 1990; Boisde & Meuly, 1993).

1.4 Occurrence

1.4.1 *Natural occurrence*

Coumarin was first isolated by Vogel in 1820 by extraction from tonka beans (*Dipteryx odorata*). It was subsequently identified in a large number of plants belonging to many different families. Its better known occurrences are in sweet clover (*Melilotus alba* and *M. officinalis*), sweet woodruff (*Asperula odorata*), vanilla leaf (*Trilisa odoratissima*), vanilla beans *(Vanilla planifolia)*, cassia (*Cinnamorum cassia*), lavender (*Lavendula officinalis*) and balsam of Peru (*Myroxylon pereirae*) (Perone, 1972; Marles *et al.*, 1987*;* Boisde & Meuly, 1993; Budavari, 1998).

Coumarin has been isolated from legumes, orchids, grasses and citrus fruits (Perone, 1972). It is found at particularly high levels in some essential oils, such as cinnamon leaf and bark oil, cassia leaf oil and lavender oil (Lake, 1999).

A broad spectrum of coumarin derivatives (present both in the free state and as glucosides) have also been found in many plants; to date at least 1300 have been identified, principally as secondary metabolites in green plants (Hoult & Payd, 1996).

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), approximately 240 000 workers in the United States were potentially exposed to coumarin. National estimates of workers potentially exposed were not available from other countries. Occupational exposure to coumarin may occur in its production and in its use in the manufacture and formulation of many products.

No other data on occupational exposures were available to the Working Group.

1.4.3 *Environmental occurrence*

Reports of the release of coumarin to the environment through various waste streams are scant. The maximum total daily human exposure to coumarin has been estimated to be 0.06 mg/kg bw, comprising 0.02 mg/kg bw per day from dietary exposure, and 0.04 mg/kg bw per day from fragrance use in cosmetic products (Lake, 1999).

(*a*) *Foods and fragrances*

Coumarin is a natural product found at high levels in some essential oils, particularly cinnamon leaf oil (40 600 ppm (mg/kg)), cinnamon bark oil (7000 ppm), other types of cinnamon (900 ppm), cassia leaf oil (17 000–87 300 ppm), peppermint oil (20 ppm), lavender oil, woodruff and sweet clover as well as in green tea (0.2–1.7 ppm), fruits such as bilberry and cloudberry and other foods such as chicory root (Boisde & Meuly, 1993; TNO, 1996; Lake, 1999). It is also found in Mexican vanilla extracts (Sullivan, 1981; Marles *et al*., 1987).

(*b*) *Medical uses*

As a consequence of its medical use, many individuals have been exposed to therapeutic doses of coumarin ranging from 100 to 7000 mg per day for periods ranging from two weeks to over two years (Marshall *et al*., 1994).

(*c*) *Personal care products*

In personal care products, usual (and maximum) concentrations were found to be for soap, 0.03% (0.2%), detergent, 0.003% (0.02%), creams and lotions, 0.015% (0.1%) and perfumes, 0.3% (0.8%) (Cohen, 1979). More recently, coumarin was found in 11 of 22 perfumes at concentrations (w/v) of 0.046–6.043% (Rastogi *et al.*, 1996) and 40 of 73 deodorants on the European market at concentrations ranging from 1 to 1411 ppm [0.0001–0.14%] (Rastogi *et al*., 1998).

Coumarin penetrates human skin rapidly and efficiently (see Section 4.1).

1.5 Regulations and guidelines

No occupational exposure limit for coumarin in workplace air has been reported.

The Council of Europe has listed coumarin as an ‘active principle’ and the maximum permitted concentrations of coumarin in foodstuffs are given in Annex II of European directive (88/388/EEC) (European Commission, 1988). The general limit for coumarin in food and non-alcoholic beverages is 2 mg/kg, while in alcoholic beverages and certain caramel confectionary products, the permitted limit is 10 mg/kg and in chewing gum it is 50 mg/kg (Lake, 1999).

Food containing any added coumarin as such or as a constituent of tonka beans or tonka extract is deemed to be ‘adulterated under the act’, based upon an order published in the Federal Register of 5 March 1954 (19 FR 1239) (Food and Drug Administration, 1999).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50–51 male and 50–51 female B6C3F_1 mice, six to seven weeks, were administered coumarin (purity, > 97%) in corn oil by gavage at doses of 0, 50, 100 and

200 mg/kg bw daily for 103 weeks. Survival of all dosed groups was similar to that of the controls. Body weight gain was reduced in high-dose females. As shown in Table 1, there was significantly increased incidence of alveolar/bronchiolar tumours (adenomas and carcinomas) at the 200 mg/kg bw dose in both males and females and increased incidence of hepatocellular adenomas in females at the low and medium doses, but not at the highest dose. There were marginal increases in the incidence of squamous-cell papillomas and carcinomas of the forestomach in the low-dose males and females (National Toxicology Program, 1993).

Groups of 52 male and 52 female Swiss CD-1 mice, four weeks of age, were administered coumarin (purity, > 98%) in the diet at concentrations of 0, 300, 1000 or 3000 mg/kg of diet (ppm) for either 101 weeks (males) or 109 weeks (females). The achieved intakes of coumarin for the three doses were 26, 86 and 280 mg/kg bw per day, respectively, for males and 28, 91 and 271 mg/kg bw per day, respectively, for females. Survival did not differ between groups, although exact rates were not recorded. Body weight gain was significantly decreased by 18% in the high-dose males and by 10% in the mid-dose males, relative to the controls. The authors reported that there was no significant increase in the incidence of pulmonary adenocarcinomas in males (11/52 control, 17/52 low-dose, 10/52 mid-dose and 20/52 high-dose) and that the incidences were within the historical control range. Although there was an increased incidence of hepatocellular tumours (adenomas and carcinomas combined) in low-dose females (0/50 in controls, 8/52 in low-dose, 4/52 in mid-dose and 3/52 in high-dose females), the authors discounted these increases as being within the historical control range for that laboratory (Carlton *et al*., 1996). [The Working Group noted that no information was provided on statistical evaluation in this paper. However, the Working Group was aware of an unpublished company report in which statistical analyses had been applied to mortality-adjusted tumour rates. The Fisher's exact test for differences between treatment groups and Mantel's test for dose-related trends showed no treatment-related effect for any tumour type.]

3.1.2 *Rat*

Coumarin was tested by oral administration in two early studies in rats (Hagan *et al.*, 1967; Bär & Griepentrog, 1967; Griepentrog, 1973) and found to produce bile duct carcinomas at high levels of exposure in the latter study (IARC, 1976). However, in a re-evaluation by other pathologists of the slides from the Bär and Griepentrog (1967) study, these lesions were deemed to be non-neoplastic cholangiofibrosis and not bile duct carcinomas (Cohen, 1979; Evans *et al*., 1989).

Groups of 50 male and 50 female Fischer 344/N rats, six to seven weeks of age, were administered coumarin (purity, > 97%) in corn oil by oral gavage at doses of 0, 25, 50 and 100 mg/kg bw daily for 103 weeks. Survival of low-, mid- and high-dose males (9/50, 2/50 and 0/50 respectively) was significantly lower ($p < 0.001$) than that of controls (28/50), as was body weight gain. In males, increased mortality was attributed

Table 1. Incidence of primary tumours in B6C3F$_1$ mice exposed to coumarin

Tumour site	Animals with tumours							
	Males				Females			
	Control	50 mg/kg bw	100 mg/kg bw	200 mg/kg bw	Control	50 mg/kg bw	100 mg/kg bw	200 mg/kg bw
Lung								
Alveolar/bronchiolar adenomas	14/50	8/50	14/50	24/51*	2/51	5/49	7/49	20/51**
Alveolar/bronchiolar carcinomas	1/50	1/50	2/50	1/51	0/51	0/49	0/49	7/51**
Liver[a]	26/50	29/50	29/50	27/51	8/50	26/49**	29/51**	12/50
Forestomach[b]	2/50	9/50*	4/50	0/51	1/52	6/50	3/51	2/51

From National Toxicology Program (1993)
[a] Hepatocellular adenomas
[b] Squamous-cell papillomas and carcinomas
* $p < 0.05$, logistic regression test
** $p < 0.01$, logistic regression test

to increased severity of age-related spontaneous chronic progressive nephropathy. As shown in Table 2, there was no increased incidence of renal tubule adenomas after conventional single-section evaluation, but the incidence was increased based on the results of step-sectioning of kidney tissue, although there was no dose–response relationship. There was an increased severity of bile duct hyperplasia and renal tubule hyperplasia in both sexes. An accompanying stop-exposure study was carried out in which groups of 20 male rats received 100 mg/kg bw per day of coumarin by gavage in corn oil for nine or 15 months followed by corn oil gavage only until the end of the study at 103 weeks. Survival was 9/20 and 2/20 in the two groups, respectively. Whereas hepatic lesions including bile duct hyperplasia were reversible and the incidence of renal tubule adenomas, based on single sections, was not significantly increased, there was an increase in the nine-month 100 mg/kg bw dose group after step-sectioning of the kidney (National Toxicology Program, 1993). [The Working Group noted that the incidence of renal tubule tumours was not increased after conventional examination of the kidney.]

Groups of 50 male and 50 female Sprague-Dawley rats were administered coumarin (purity, > 98%) in the diet at concentrations of 0, 333, 1000, 2000, 3000 and 5000 mg/kg of diet (ppm) for 104 weeks (males) or 110 weeks (females). Groups receiving 3000 and 5000 ppm were 21–28 days of age at the beginning of the study, while the other treated groups were exposed to coumarin diet *in utero* and throughout the postnatal and chronic periods. The achieved intakes of coumarin for the five doses were 13, 42, 87, 130 and 234 mg/kg bw per day, respectively, for males and 16, 50, 107, 156 and 283 mg/kg bw per day, respectively, for females. Survival was less than 50% in all groups (including controls) except the groups receiving the two highest doses, in which it was between 50 and 70%. Dose-related decreases in body weight gain in excess of 10–15% occurred in the 2000-, 3000- and 5000-ppm dose groups. Significantly increased incidences of cholangiocarcinomas, some of which were reported as metastasizing, were found in the highest-dose males and females (males: 0/65, 0/65, 0/65, 0/65, 1/65 and 37/65; females: 0/65, 0/65, 0/65, 0/65, 0/65 and 29/65, in the control, 333-, 1000-, 2000-, 3000- and 5000-ppm dose groups respectively). Hepatocellular tumours were also found in high-dose males: 2/65, 2/65, 1/65, 1/65, 6/65 and 29/65 and females: 0/65, 0/65, 0/65, 0/65, 1/65 and 12/65 (Carlton *et al*., 1996). [The Working Group noted the unusually sharp increase in tumour incidence in the liver at only the highest of five doses and the lack of adequate histopathological description of both tumour types. Given the issue related to misdiagnosis of bile duct tumours in an earlier study, the Working Group was concerned that no descriptive information or illustrations were provided to confirm the diagnosis of cholangiocarcinoma, nor a discussion of the pathology.]

3.1.3 *Hamster*

Groups of 11 or 12 male and 10–13 female Syrian golden hamsters, eight weeks old, were administered coumarin (purity, 99%) in the diet at concentrations of 0, 0.1 and 0.5% for up to two years. Survival was poor in all groups receiving coumarin, and

Table 2. Incidence of primary tumours in Fischer 344/N rats exposed to coumarin

Tumour site	Animals with tumours							
	Males				Females			
	Control	25 mg/kg bw	50 mg/kg bw	100 mg/kg bw	Control	25 mg/kg bw	50 mg/kg bw	100 mg/kg bw
Kidney[a], single section	1/49	2/50	2/51	1/50	0/49	0/50	0/50	2/49
step sections	0/49	4/50	5/51*	4/50	0/49	0/50	1/50	1/49

From National Toxicology Program (1993)
[a] Renal tubule adenoma
* $p < 0.05$, logistic regression test

in the control females. There was no evidence of increased bile duct hyperplasia, cholangiofibrosis or cholangiocarcinoma due to coumarin exposure or tumours at other sites (Ueno & Hirono, 1981). [The Working Group noted the small number of animals and the poor survival, making the study inadequate for evaluation.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

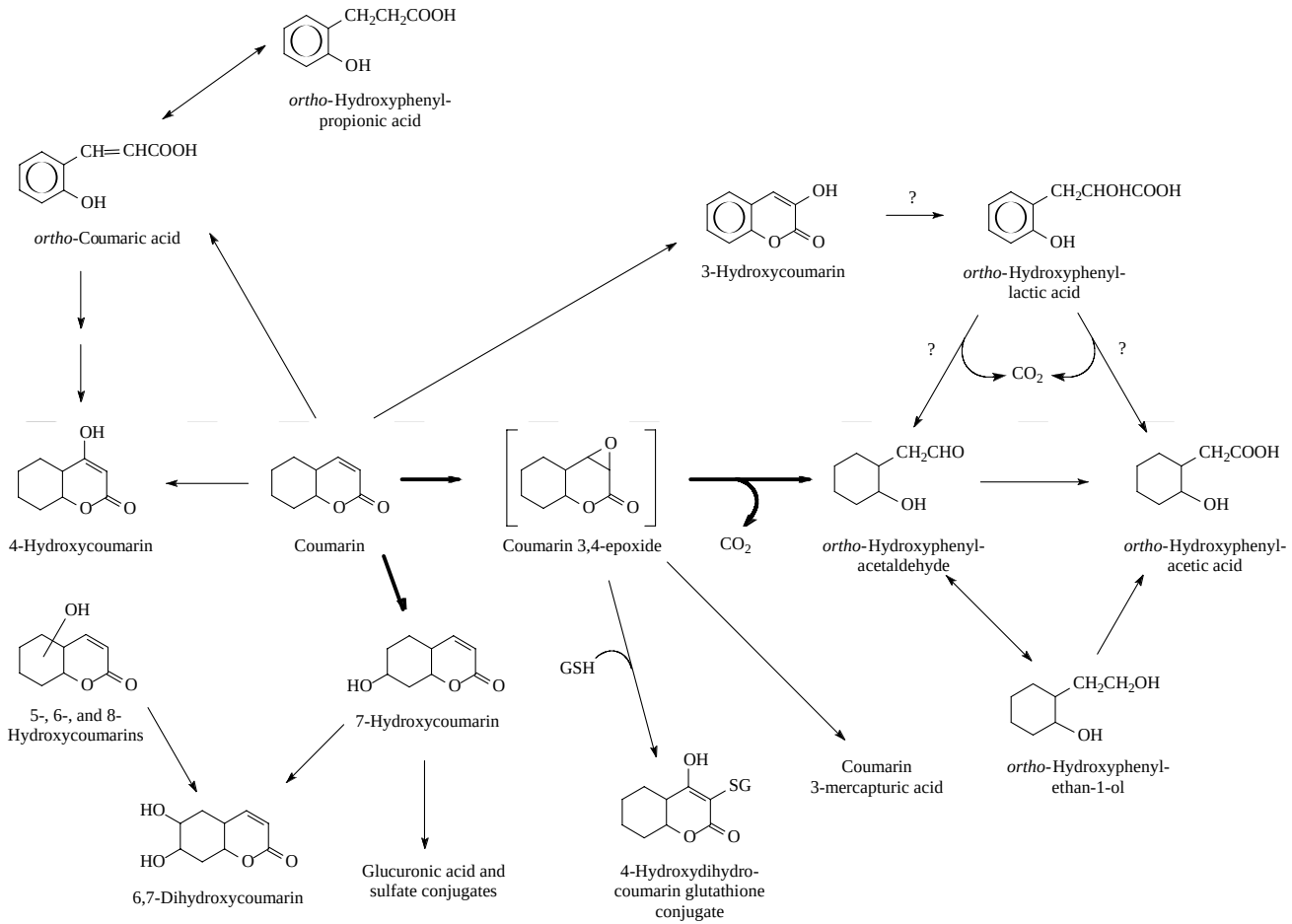
The absorption, distribution, metabolism and excretion of coumarin in humans have been reviewed (Cohen, 1979; Fentem & Fry, 1993; Pelkonen *et al*., 1997; Lake, 1999).

Toxicokinetic studies in humans have demonstrated that coumarin is rapidly absorbed from the gastrointestinal tract after oral administration and extensively metabolized by the liver in the first pass, with only 2–6% reaching the systemic circulation intact (Ritschel *et al*., 1977, 1979; Ritschel & Hoffmann, 1981).The elimination of coumarin from the systemic circulation is rapid, the half-lives following intravenous doses of 0.125, 0.2 and 0.25 mg/kg bw being 1.82, 1.46 and 1.49 h [109, 88 and 89 min], respectively (Ritschel *et al.*,1976). Coumarin is also extensively absorbed after dermal application. In one study with human subjects, some 60% of a 2.0-mg dose applied for 6 h was absorbed (reviewed in Lake, 1999). The percutaneous absorption of coumarin has also been demonstrated *in vitro* with human skin (Beckley-Kartey *et al.*, 1997; Yourick & Bronaugh, 1997).

The rapid excretion of coumarin, primarily as 7-hydroxycoumarin conjugates, in the urine of human subjects given coumarin orally suggests that there is little or no biliary excretion of coumarin metabolites in humans (Shilling *et al*., 1969; Cholerton *et al*., 1992; Egan & O'Kennedy, 1992; Rautio *et al*., 1992; Iscan *et al*., 1994).

Coumarin exhibits marked species differences in its metabolism (Cohen, 1979; Fentem & Fry, 1993; Lake, 1999). The major primary pathways of coumarin metabolism are 7-hydroxylation or metabolism of the lactone ring by ring opening and cleavage at carbon atom 2 to yield carbon dioxide. The first step in the latter pathway is the formation of the unstable coumarin 3,4-epoxide which degrades spontaneously to form *ortho*-hydroxyphenylacetaldehyde and may be subsequently converted to *ortho*-hydroxyphenylethanol and *ortho*-hydroxyphenylacetic acid. Coumarin may also be metabolized by hydroxylation to yield 3-, 4-, 5-, 6- or 8-hydroxycoumarin and 6,7-dihydroxycoumarin and, by opening of the lactone ring, to yield *ortho*-coumaric acid (*ortho*- hydroxyphenylcinnamic acid) and *ortho*-hydroxyphenylpropionic acid (Norman & Wood, 1984; Fentem *et al.*, 1991; Lake *et al*., 1992a,b; Born *et al*., 1997). The pathways of coumarin metabolism are shown in Figure 1.

Figure 1. Metabolic pathways of coumarin in animals and humans



Adapted from Lake (1999)

The major pathway of coumarin metabolism in most human subjects is 7-hydroxylation to form 7-hydroxycoumarin, which is excreted in the urine as both glucuronic acid and sulfate conjugates. Coumarin 7-hydroxylation activity exhibits a Gaussian distribution in Caucasian populations (Cholerton *et al.*, 1992; Rautio *et al.*, 1992), but some individuals are deficient in this activity.

Hadidi *et al.* (1997) gave members of a family 2 mg coumarin orally and collected their urine for 8 h. One subject excreted < 0.03% of the dose as 7-hydroxycoumarin and 50% as *ortho*-hydroxyphenylacetic acid, but three others excreted mainly 7-hydroxycoumarin (> 41% of dose) and 4–10% as *ortho*-hydroxyphenylacetic acid. Oscarson *et al.* (1998) refer to two individuals (among a population of two hundred) who were totally deficient in 7-hydroxycoumarin excretion after an oral dose of 5 mg coumarin.

CYP2A6 (cytochrome P450 2A6) has been purified from human liver and CYP2A6 cDNA expression systems are available. Many studies have demonstrated marked interindividual variation in the levels of hepatic CYP2A6 protein, mRNA and associated microsomal coumarin 7-hydroxylase activity (reviewed in Pelkonen *et al*., 1997; Lake, 1999). The role of CYP2A6 in the metabolism of coumarin by human liver microsomes has been confirmed by Sai *et al.* (1999), who found that a monoclonal antibody to CYP2A6 inhibited coumarin 7-hydroxylation by more than 94%.

The marked interindividual variation in coumarin metabolism to 7-hydroxycoumarin has led to studies to evaluate whether a genetic polymorphism exists in human CYP2A6.

The occurrence of variant alleles in the human *CYP2A6* gene was shown by Fernandez-Salguero *et al.* (1995); these were designated *CYP2A6*1* (wild type), *CYP2A6*2* and *CYP2A6*3*. *CYP2A6*2* has a point mutation in codon 160 and the resulting protein product is unable to 7-hydroxylate coumarin (Fernandez-Salguero *et al.*, 1995; Gullstén *et al.*, 1997; Hadidi *et al.*, 1997). The functional significance of the rare *CYP2A6*3* allele is uncertain. The population frequency of these mutant alleles is uncertain at present; initial claims that the incidence of the *CYP2A6*2* allele is 4–17% of European populations (Fernandez-Salguero *et al.*, 1995; Gullstén *et al.*, 1997) have been challenged by Oscarson *et al.* (1998), who found the incidence to be 1–3%. These authors highlighted methodological uncertainties in polymerase chain reaction-based genotyping procedures. Establishment of the significance of the genetic polymorphism in *CYP2A6* must await definitive genotyping and phenotyping procedures.

While 7-hydroxylation is the major metabolic pathway of coumarin in most subjects, humans also convert coumarin to *ortho*-hydroxyphenylacetic acid.

Shilling *et al*. (1969) reported that after an oral dose of 200 mg coumarin per subject, while 7-hydroxycoumarin accounted for 79% of the excreted dose (range, 68–92%), a further 4% of the dose (range, 1–6%) was present in the first 24-h urine as *ortho*-hydroxyphenylacetic acid.

Ten human subjects received coumarin orally (1 g and 2 g) and intravenously (250 mg) in a randomized cross-over study and their 0–48 h urines were assayed for *ortho*-hydroxyphenylacetic acid. After intravenous administration, 1.5% (range, 0.3–3.6%)

of the dose was recovered as *ortho*-hydroxyphenylacetic acid, while after oral administration the recoveries were 3.5% (1.8–7.0%) and 5.1% (1.1–13.5%) after the 1-g and 2-g doses, respectively (Meineke *et al.*, 1998).

4.1.2 *Experimental systems*

The toxicokinetics of coumarin have been studied in a number of species including rats (intraperitoneal, intravenous, oral and topical administration) (Hardt & Ritschel, 1983; Ritschel & Hussain, 1988), dogs (intravenous and oral) (Ritschel & Grummich, 1981), gerbils (intraperitoneal) (Ritschel & Hardt, 1983) and rhesus monkeys (intravenous and oral) (Ritschel *et al.*, 1988). Generally, the half-life for the elimination of coumarin is similar in all species examined, being around 1–4 h (Lake, 1999). In rats, the toxicokinetics of coumarin are non-linear at intraperitoneal doses greater than 10 mg/kg bw (Hardt & Ritschel, 1983).

Following oral administration, coumarin is rapidly absorbed from the gastrointestinal tract and is distributed throughout the body (Cohen, 1979; Fentem & Fry, 1993; Pelkonen *et al.*, 1997; Lake, 1999). The compound is extensively metabolized in all species, with little excretion of unchanged coumarin. No significant tissue accumulation of coumarin and/or coumarin metabolites occurred after oral administration to rats and rabbits (Kaighen & Williams, 1961) or intraperitoneal administration to rats (van Sumere & Teuchy, 1971).

There are important quantitative differences between species in the routes of elimination of coumarin metabolites. In rats, biliary excretion occurs with an appreciable proportion of the dose excreted in the faeces (Cohen, 1979; Lake, 1999). For example, after a 50-mg/kg bw oral or intraperitoneal dose of coumarin to rats, some 50% was excreted in the bile as unknown metabolites within 24 h (Williams *et al*., 1965). The urine appears to be the major route of coumarin excretion in Syrian hamsters, rabbits and baboons, but not in marmosets (Kaighen & Williams, 1961; Waller & Chasseaud, 1981; Lake *et al.*, 1989a, 1990; Lake, 1999).

Unlike in humans, the major metabolic pathway of coumarin in rats is the 3,4-epoxidation pathway. After a 100-mg/kg bw oral dose of [3-^{14}C]coumarin, urinary 3-hydroxycoumarin, 7-hydroxycoumarin, *ortho*-hydroxyphenyllactic acid and *ortho*-hydroxyphenylacetic acid accounted for 1.8, 0.4, 0.8 and 20% of the dose, respectively. Various metabolites including *ortho*-hydroxyphenylacetic acid were detected in the faeces (Kaighen & Williams, 1961). Other studies *in vivo* have confirmed that rats are poor 7-hydroxylators of coumarin, with urinary 7-hydroxycoumarin accounting for < 1% of the dose (van Sumere & Teuchy, 1971; Lake *et al*., 1989a).

Because of the relative ease of measurement of 7-hydroxycoumarin, many studies have examined this pathway of coumarin metabolism after oral administration. Overall, several species including rats, most mouse strains, Syrian hamsters, guinea-pigs, dogs, marmosets and squirrel monkeys are poor 7-hydroxylators, excreting ≤ 5% of the administered dose as urinary 7-hydroxycoumarin (Cohen, 1979; Lake, 1999).

Certain mouse strains, such as DBA/2 and 129/Rr strains, excrete up to 26% of an intraperitoneally administered dose of coumarin as 7-hydroxycoumarin (Lush & Andrews, 1978). Species such as rabbits, cats and pigs have been reported to excrete 12–19% of the dose as urinary 7-hydroxycoumarin (Kaighen & Williams, 1961; Gangolli *et al*., 1974; Lake, 1999). In contrast, baboons, like humans, are extensive 7-hydroxylators of coumarin, excreting 60–66% of a dose of coumarin as urinary 7-hydroxycoumarin (Gangolli *et al.*, 1974; Waller & Chasseaud, 1981).

Apart from glucuronic acid and sulfate conjugation of hydroxycoumarins, other phase II pathways of coumarin metabolism have been identified. For example, *ortho*-coumaric acid may be conjugated with glycine (Lake, 1999), and a coumarin mercapturic acid conjugate has also been reported (Huwer *et al*., 1991). Coumarin may also be metabolized by the gastrointestinal microflora to 3,4-dihydrocoumarin and *ortho*-hydroxyphenylpropionic acid under anaerobic conditions (Scheline, 1968).

Coumarin metabolism has been studied *in vitro* in liver microsomes, hepatocytes and liver slices. Metabolites of coumarin detected in such systems include all six hydroxycoumarins, *ortho*-hydroxyphenylacetaldehyde, *ortho*-hydroxyphenylethanol, *ortho*-hydroxyphenylacetic acid, *ortho*-hydroxyphenyllactic acid, *ortho*-hydroxyphenylpropionic acid, *ortho*-coumaric acid and 6,7-dihydroxycoumarin (Lake, 1999). Studies with radiolabelled coumarin have demonstrated that coumarin can be converted to metabolite(s) that can bind covalently to proteins (Lake, 1984). In keeping with in-vivo studies, 7-hydroxycoumarin formation is only a minor pathway of metabolism in most species. Coumarin metabolism was examined in hepatic microsomes from eight animal species and humans. The overall rate of metabolism varied from 6.7 to 0.11 nmol/mg protein/min, in the rank order hamster>rat>mouse, cynomolgus monkey, human, rabbit>guinea-pig, dog>>cat. The contribution of 7-hydroxylation to the metabolism varied even more, by 225-fold (from 8 to 1800 pmol/mg protein/min for cat and monkey microsomes, respectively, accounting for 84% of total metabolism in human and 73% in monkey, but only 7% in cat, 3% in mouse and 0.3% in rat (Pearce *et al.*, 1992). Depending on the substrate concentration employed, *ortho*-hydroxyphenylacetaldehyde can be a major metabolite of coumarin in liver microsomes from rats, mice, Syrian hamsters and gerbils (Fentem & Fry, 1992; Lake *et al.*, 1992a,b). In mice, marked strain differences have been observed, with high coumarin 7-hydroxylase activity in female mice from strains such as DBA/2 and 129/J (Negishi *et al.*, 1989; Lovell *et al.*, 1999). However, the major pathway of coumarin metabolism in mouse remains 3,4-epoxidation, even in strains with high 7-hydroxylase activity (Lovell *et al*., 1999). The CYP isoform responsible for coumarin 7-hydroxylation in mouse liver is CYP2A5 and both baboons and cynomolgus monkeys possess hepatic CYP2A isoforms with properties similar to those of human CYP2A6 (Pelkonen *et al*., 1997; Lake, 1999).

It is of interest that studies with cloned human cytochromes P450 expressed in insect SF9 cells have shown that only CYP2A6 catalysed 7-hydroxylation, while the formation of *ortho*-hydroxyphenylacetaldehyde was supported by CYP1A1, 1A2, 2B6, 2E1 and 3A4 (Zhuo *et al.*, 1999).

4.2 Toxic effects

4.2.1 *Humans*

Recommended doses of coumarin in clinical medicine range from 8 mg per day for the treatment of venous constriction to 7000 mg per day in antineoplastic therapies (Marshall *et al*., 1994; Lake, 1999). While various mild side-effects have been reported following coumarin treatment, alterations in liver function have been noted in only a small proportion of patients. Reports of overt toxicity are rare (Cox *et al*., 1989).

Casley-Smith and Casley-Smith (1995) reported two cases of hepatotoxicity in five trials involving 1106 lymphoedema patients taking 400 mg coumarin daily for a mean duration of 14.6 months. This incidence is similar to that reported by Cox *et al*. (1989) in trials with 2173 patients with cancer or chronic infections. The majority of these patients received 100 mg coumarin per day for one month followed by 50 mg per day for two years. Only eight patients (0.37%) developed elevated serum aminotransferase levels (peak levels of 360–696 I.U./L) after total coumarin doses between 1 and 15 g. No evidence of liver toxicity was observed in 45 renal-cell carcinoma patients given 100 mg coumarin daily in combination with cimetidine (Marshall *et al*., 1987a). In other studies with similar dosing regimens, no evidence of liver toxicity was found in groups of 17 (Nolte *et al*., 1987), 22 (Marshall *et al*., 1989), 24 (Marshall *et al*., 1987b) and 50 (Dexeus *et al*., 1990) cancer patients. No evidence of liver toxicity was reported in extensive trials with lymphoedema patients given 400 mg coumarin per day in combination with diethylcarbamazine (Jamal *et al*., 1989).

Some cases of hepatotoxicity have been reported to be associated with exposure to coumarin. One possible case was reported by Beinssen (1994) and six by Loprinzi *et al*. (1997). Marshall *et al*. (1994) reported one case in which elevated serum aminotransferase levels were measured in a patient given 5 g coumarin per day. In two lymphoedema patients given 90 mg coumarin per day for five months, Koch *et al*. (1997) reported elevated serum alanine aminotransferase activity. Faurschou (1982) reported a case of toxic hepatitis in a patient given coumarin daily for eight weeks, which was characterized by hepatomegaly and elevated serum enzyme levels. All signs of liver toxicity returned to normal on cessation of treatment.

Coumarin was not toxic at concentrations up to 100 μg/mL to human peripheral blood mononuclear cells *in vitro*. Coumarin and its metabolite, 7-hydroxycoumarin, produced significant suppression of human bone marrow progenitor stem cell activity at concentrations greater than 200 μg/mL, whereas coumarin concentrations of 25 μg/mL and above produced suppression of murine bone marrow progenitor stem cell activity (Gallicchio *et al*., 1989).

In various human tumour cell lines (lymphoblastic cell line, CCRF CEM; gastric carcinoma cell line, St 23132; hepatoma-derived cell line, HepG2; colon-carcinoma cell line, Caco-2) coumarin [IC_{50} values ranging from 232 to 522 μg/mL] and its major metabolite 7-hydroxycoumarin [IC_{50} values ranging from 110 to 436 μg/mL] inhibited

cell proliferation after 48 h incubation. The glucuronide of 7-hydroxycoumarin (tested up to 2315 μg/mL) did not produce this response (Weber *et al*., 1998).

4.2.2 *Experimental systems*

(*a*) *Single-dose studies*

The acute oral LD_{50} of coumarin has been reported to be 420 mg/kg bw in C3H/HeJ mice and 780 mg/kg bw in DBA/2J mice (Endell & Seidel, 1978). In three mouse strains, the oral LD_{50} of coumarin has been reported to range from 196 to 780 mg/kg bw (reviewed in Cohen, 1979; Egan *et al*., 1990). The oral LD_{50} for coumarin in various strains of rats is reported to be 292–680 mg/kg bw (Hazleton *et al*., 1956).

In male Sprague-Dawley or Wistar rats, single oral or intraperitoneal doses of coumarin ranging from 125 to 500 mg/kg bw resulted in rapid depletion of hepatic non-protein sulfhydryl groups, primarily glutathione. After 24 h, centrilobular hepatic necrosis was observed together with dose-dependent elevation in plasma transaminase activities (Lake, 1984; Lake *et al*., 1989b; Fentem *et al*., 1992; Lake & Evans, 1993; Lake *et al*., 1994). Studies with various modulators of cytochrome P450 activity and with depletors of glutathione levels demonstrated that coumarin-induced hepatotoxicity in the rat is likely to be mediated via one or more reactive metabolites generated by cytochrome P450-dependent enzymes and that glutathione conjugation constitutes a detoxification pathway (Lake, 1984, 1999). Lake *et al*. (1989b) showed that dihydrocoumarin, which lacks the 3,4-double bond, produced little hepatotoxicity in male Sprague-Dawley rats *in vivo* (127 and 254 mg/kg bw intraperitoneally). Subsequently, Lake *et al*. (1994) demonstrated that coumarin (intraperitoneally, 0.86 and 1.71 mmol/kg bw), but none of the coumarin methyl derivatives examined (intraperitoneally, 1.71 and/or 2.57 mmol/kg bw), produced dose-related hepatic necrosis in male Sprague-Dawley rats.

In male Wistar rats, coumarin, 3-methylcoumarin or 4-methylcoumarin was administered intraperitoneally at a single dose of 1.03 mmol/kg bw and rats were killed 24 h later. Coumarin produced histological evidence of centrilobular necrosis, while the methyl analogues were much less toxic at equivalent doses. In the same study, these compounds had the same order of cytotoxicity in isolated hepatocytes as that observed *in vivo* (Fernyhough *et al*., 1994).

Cottrell *et al.* (1996) reported that a single oral dose of coumarin produced liver necrosis in mice; 200 mg/kg bw coumarin was hepatotoxic to both C3H/He and DBA/2 mice. Hepatotoxicity was characterized by an increase in plasma aminotransferase activity, mild subcapsular linear hepatocyte necrosis and, in some C3H/He mice, centrilobular necrosis. Mice were pretreated with β-naphthoflavone (80 mg/kg bw), Aroclor 1254 (54, 125 or 162 mg/kg bw) or vehicle alone by intraperitoneal injection for three consecutive days. Twenty-four hours later, a single dose of coumarin (200 mg/kg bw) or vehicle was administered by gavage. Pretreatment with

β-naphthoflavone or Aroclor 1254 did not significantly alter coumarin hepatotoxicity in C3H/He mice, while hepatic microsome metabolism of [^{14}C]coumarin doubled following administration of either inducer. In DBA/2 mice, pretreatment with either β-naphthoflavone or Aroclor 1254 did not affect coumarin-induced hepatotoxicity.

The toxicity of coumarin has also been assessed in other species. A single intraperitoneal injection of coumarin (125 mg/kg bw) caused centrilobular necrosis in Wistar rats but not in Mongolian gerbils (Fentem *et al*., 1992). Coumarin has been reported to be hepatotoxic and nephrotoxic in dogs at an oral dose of 100 mg/kg bw given for periods of 8–22 days (Hazleton *et al*., 1956; Hagan *et al*., 1967). Hepatotoxic effects were also noted in dogs given 25 or 50 mg/kg bw coumarin for longer periods, but not in dogs given 10 mg/kg bw coumarin for up to 350 days (Hagan *et al*., 1967).

A significant reduction in CYP2A and CYP2G levels in the olfactory mucosa of Wistar rats and C57BL/6 mice and suppression of nasal CYP2A in rats occurred within 48 h following a single intraperitoneal injection of coumarin (50 mg/kg bw). The decrease in cytochrome P450 levels was accompanied by necrosis, cell loss and basal cell metaplasia in the olfactory mucosa. Neither 7-hydroxycoumarin nor 3,4-dihydrocoumarin at a dose of 50 mg/kg bw depleted nasal cytochromes P450 (Gu *et al*., 1997).

Coumarin produces pulmonary toxicity in mice. Male and female B6C3F$_1$ mice were dosed orally by gavage with 0, 10, 20, 50, 100, 150 or 200 mg/kg bw coumarin and lungs were evaluated histologically 24 h later. At doses of 150 mg/kg bw or greater, coumarin caused selective injury to the Clara cells in the distal bronchiolar epithelium. The time course of this injury and recovery of the cells was studied up to seven days following a single dose of 200 mg/kg bw coumarin. At 24–48 h, Clara cells were observed sloughed into the lumen of the terminal bronchioles along with thinning of the epithelium and flattening of the remaining ciliated cells. By seven days after dosing, the Clara cells had regenerated and the bronchiolar epithelium appeared normal. In male Fischer 344 rats dosed by gavage (200 mg/kg bw; single dose), Clara cell toxicity was not observed in the distal bronchioles. However, coumarin caused generalized epithelial necrosis in the upper airways of rats involving both ciliated and non-ciliated cells. Oral administration of coumarin (200 mg/kg bw; single dose) to female mice and male rats resulted in no evidence of hepatocellular necrosis in mice, whereas marked centrilobular necrosis was observed in the rat liver. Clinical markers of hepatic injury (aminotransferase and succinate dehydrogenase activity) were increased nearly 100-fold in rats and 2- to 3-fold in mice 24 h after treatment (Born *et al.*, 1998).

(*b*) *Multiple-dose studies*

A number of studies have examined the hepatic biochemical and morphological changes in rats produced by coumarin administration for periods ranging from one week to two years (reviewed in Lake, 1999). Coumarin administration results in increased liver weight and in a variety of morphological changes in treated rats that are dependent

on the magnitude of the dose and the duration of treatment. Such changes include necrosis, apoptosis, vacuolation, fatty change and bile duct hyperplasia. While centrilobular hepatic necrosis may be observed in rats treated with coumarin for at least four weeks, this lesion tends to regress with prolonged treatment in favour of the development of bile duct hyperplasia and cholangiofibrosis in the periportal area of the liver lobule (Grasso *et al.*, 1974; Evans *et al.*, 1989; Lake & Grasso, 1996). Kidney lesions were not observed in studies with Sprague-Dawley and Osborne-Mendel rats chronically fed coumarin at dietary levels of up to 0.5% (Hagan *et al.*, 1967; Carlton *et al.*, 1996).

Over a 16-day period, coumarin was administered by gavage to groups of five male and five female Fischer 344 rats and $B6C3F_1$ mice at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw or 0, 40, 75, 150, 300, or 600 mg/kg bw, respectively, on five days per week for a total of 12 treatment days. All female and four male rats receiving 400 mg/kg bw coumarin died. There were no clinical signs of organ-specific toxicity in rats. Clinical findings in mice included inactivity, excessive lacrimation, piloerection, bradypnoea, ptosis or ataxia. All the mice receiving 600 mg/kg bw coumarin died, together with one male and one female in the 300-mg/kg bw groups and one male in the 75-mg/kg bw group. No histopathological examination was performed in this study (National Toxicology Program, 1993).

When $B6C3F_1$ mice were treated orally for five days with 200 mg/kg bw coumarin, mice became tolerant to the coumarin-induced swelling of Clara cells and necrosis in the terminal bronchioles which is observed after a single dose of coumarin (200 mg/kg bw). Although after five days of coumarin treatment, there were still areas of bronchiolar epithelial flattening and hyperplasia, by 10 days mouse lungs appeared to be normal. Together with Clara cell necrosis (following a single dose), the levels of cytochromes P450 such as CYP2A4/5, CYP2B10, CYP2C29, CYP2E1 and CYP2F2 were decreased. In tolerant mice, the levels of these enzymes returned to normal (Born *et al.*, 1999).

Coumarin administration in the diet (0.25–0.5%) of male Wistar rats and female ICR/Ha mice for two weeks induced glutathione peroxidase activity in the stomach (1.7-fold) and glutathione *S*-transferase in the liver (5.3-fold), respectively (Sparnins *et al.*, 1982; van Lieshout *et al.*, 1998).

In a 13-week study, groups of ten Fischer 344 rats and ten $B6C3F_1$ mice were administered coumarin by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw. Three male and three female rats and two male mice receiving 300 mg/kg bw coumarin died. Absolute and relative liver weights increased in male and female rats and mice treated with 150 or 300 mg/kg bw coumarin. At the same doses, centrilobular hepatocellular degeneration and necrosis were observed in rats along with chronic active inflammation and bile duct hyperplasia. A re-evaluation of the kidneys revealed minimal nephropathy with tubular casts in the high-dose male and female rats and minimal to marked focal necrosis of proximal convoluted tubule epithelium was also observed. In coumarin-treated mice (300 mg/kg bw), centrilobular hepatocellular hypertrophy was observed (National Toxicology Program, 1993).

In one-, four- and 13-week studies, the effects of coumarin treatment were compared in male Sprague-Dawley rats, CD-1 mice and Syrian hamsters. Rats were fed 0–0.75% coumarin for one and four weeks and 0.5% coumarin for 13 weeks. Mice and hamsters were fed 0–0.5 and 0–1.0% coumarin, respectively, for one, four or 13 weeks. In the rat, coumarin caused dose-related hepatotoxic effects which included vacuolar degeneration, apoptosis and bile duct proliferation and increases in serum bilirubin content and both serum and hepatic γ-glutamyltranspeptidase activity. A sustained stimulation of hepatocyte replicative DNA synthesis was observed in rats treated for four and 13 weeks. Levels of total hepatic glutathione were increased approximately twofold, and there were statistically significant decreases in microsomal cytochrome P450 content and ethylmorphine *N*-demethylase activity. These effects were reduced or not observed in mice and hamsters (Lake & Grasso, 1996).

In a two-year study in which male and female Syrian hamsters were fed coumarin at dietary levels of 0.1 and 0.5%, no evidence of any coumarin-induced liver lesion was observed (Ueno & Hirono, 1981).

Coumarin was administered in the diet for two years to Sprague-Dawley rats and CD-1 mice in a study by Carlton *et al.* (1996) which is described more fully in Section 3.1.2. Histopathological evidence of hepatotoxicity was observed in rats, with males affected more severely than females. No dose-related clinical signs were observed in the CD-1 mice nor were there any changes in clinical pathology, haematology or microscopic pathology (Carlton *et al.*, 1996).

Coumarin was administered by oral gavage for two years to male and female Fischer 344 rats and B6C3F_1 mice, at doses of 0, 25, 50, and 100 mg/kg bw and 0, 50, 100, and 200 mg/kg bw, respectively (see Section 3.1.2). Hepatic lesions consisted of hepatocellular necrosis, fibrosis, cytological alteration and increased severity of bile duct hyperplasia. The incidence of forestomach ulcers was significantly greater in treated rats than in controls. Administration of coumarin to mice was associated with centrilobular hypertrophy, syncytial alteration and eosinophilic foci in the liver. No non-neoplastic lesions of the lungs of dosed mice were considered to be chemical-related (National Toxicology Program, 1993).

Administration of coumarin to baboons at dose levels of 0, 2.5, 7.5, 22.5 or 67.5 mg/kg bw per day in the diet for 16–24 months resulted in increased relative liver weight only at the highest dose level. While light microscopic examination of liver sections revealed no abnormalities, ultrastructural examination revealed a dilatation of the endoplasmic reticulum in three of four baboons given 67.5 mg/kg per day coumarin (Evans *et al.*, 1979).

(*c*) *In-vitro studies and species comparisons*

Lake *et al.* (1989b) compared the toxicity of coumarin and selected coumarin metabolites, including 7-hydroxycoumarin, 3-hydroxycoumarin and *ortho*-hydroxyphenylacetic acid, in metabolically-competent rat hepatocytes. Coumarin was more toxic than

any of its metabolites. The IC_{50} value for inhibition of protein synthesis was approximately 0.5 mmol/L for coumarin, whereas the 7-hydroxy and 3-hydroxy metabolites had IC_{50} values greater than 1 mmol/L, with *ortho*-hydroxyphenylacetic acid being essentially non-toxic.

Ratanasavanh *et al*. (1996) prepared hepatocytes from male Sprague-Dawley rats, DBA/2J mice, Fauve de Bourgogne rabbits and humans. The hepatocytes were incubated with 0.1, 0.25 or 0.5 mmol/L coumarin (and 1 mmol/L for human hepatocytes). Coumarin was hepatotoxic in cells prepared from rats, mice and rabbits, as judged by cell morphology and by lactate dehydrogenase release. Human hepatocytes were sensitive to coumarin toxicity only at a concentration of 1 mmol/L.

Price *et al*. (1996) compared the toxicity of 0.5, 1 and 2 mmol/L coumarin in 24 h cultured precision-cut male Sprague-Dawley rat, Dunkin-Hartley guinea-pig, cynomolgus monkey and human liver slices. Coumarin toxicity, based on liver protein synthesis and potassium content, was concentration-dependent in rat and guinea-pig liver, whereas monkey and human liver were relatively resistant.

Born *et al*. (2000) demonstrated that *ortho*-hydroxyphenylacetaldehyde (4 mmol/L) was much more cytotoxic than coumarin (4 mmol/L) to Chinese hamster ovary cells K_1, a cell line that does not contain cytochromes P450. When both of these compounds were investigated in metabolically active hepatocytes isolated from male Sprague-Dawley rats, *ortho*-hydroxyphenylacetaldehyde (0.8 mmol/L) caused a greater cytotoxic response compared with coumarin (0.8 mmol/L). 3-Hydroxycoumarin (0.8 mmol/L), not a product of coumarin epoxidation, did not cause a change in cell viability or an increase in lactate dehydrogenase activity.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 26–30 female NMRI mice were fed diets containing 0, 0.05, 0.1 and 0.25% coumarin on days 6–17 of gestation. There was no effect on the total number of implantations or on the proportions that were resorptions or fetal deaths, nor any reduction in fetal weight (Roll & Bär, 1967).

No embryotoxic or fetotoxic effects were seen in either rats, rabbits or miniature pigs given 10–100-fold the human therapeutic dose of coumarin plus rutin (Grote & Günther, 1971; Grote & Weinmann, 1973; Grote *et al.*, 1977).

Coumarin was one of a series of chemicals used in an assessment of the predictability of two in-vitro assays for mammalian teratogenesis. The assays were the human epithelial palatal mesenchymal (HEPM) cell assay, which evaluates effects on proliferative potential, and the mouse ovarian tumour (MOT) cell assay, which evaluates

the effect on attachment of ascites tumour cells to concanavalin-coated beads. The IC_{50} values for coumarin in both assays were in excess of 1 mmol/L, and the authors considered the results to be negative (Steele *et al*., 1988).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Genotoxicity data on coumarin have been recently reviewed (Lake, 1999).

Coumarin induced gene mutations in *Salmonella typhimurium* strain TA100 with metabolic activation. A positive result was also reported in *Salmonella* strain TA7002, which responds specifically to reversions at a site where the change is T:A to A:T. However, coumarin failed to induce gene mutations in other strains of this type. Coumarin induced sister chromatid exchanges in Chinese hamster cells *in vitro* only without exogenous metabolic activation, and chromosomal aberrations only in the presence of exogenous metabolic activation. It did not induce micronuclei in rat primary hepatocytes, although micronuclei were found in established human hepatoma cells treated with coumarin. The two positive chromosome responses (aberrations, micronuclei) occurred only at the highest dose tested, and there was no clear dose–response relationship. Coumarin failed to induce gene mutations at the *Hprt* locus in Chinese hamster ovary cells. It did not induce unscheduled DNA synthesis in human liver slices *in vitro*.

Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*. Coumarin did not induce either micronuclei in mice or unscheduled DNA synthesis in rats *in vivo*.

Coumarin modulates the mutagenic effects of other chemicals such as aflatoxin B_1 and a range of heterocyclic amines, and of physical agents such as ultraviolet light, and has been generally considered to be an antimutagen (Ohta *et al*., 1983; Sanyal *et al*., 1997; Goeger *et al.*, 1998; Simic *et al*., 1998). However, there are reports that it also acts as a co-mutagen in some assays (Goeger *et al*., 1999).

4.5 Mechanistic considerations

Marked inter-species differences have been observed in the metabolism and toxicity of coumarin. The metabolism of coumarin involves two primary pathways, 7-hydroxylation and ring-opening to *ortho*-hydroxyphenylacetaldehyde. Coumarin is hepatotoxic in rat, mouse and dog, species in which ring-opening predominates. In contrast, humans and baboons, in which 7-hydroxylation is most evident, rarely show

Table 3. Genetic and related effects of coumarin

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	–	+[c]	1000 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate[d]	Haworth *et al.* (1983)
Salmonella typhimurium TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, Mix[e], reverse mutation	–	–	1000 µg/mL	Gee *et al*. (1998)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		500 µg/mL; inj	Yoon *et al.* (1985)
Gene mutation, K_1BH_4 Chinese hamster ovary cells *Hprt* locus *in vitro*	–	–	500 µM	Goeger *et al*. (1999)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	–	100	National Toxicology Program (1993)
Micronucleus formation, primary rat hepatocytes *in vitro*	–	NT	73	Müller-Tegethoff *et al*. (1995)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	(+)	1600	National Toxicology Program (1993)
Micronucleus formation, human Hep-G2 cells *in vitro*	(+)	NT	500	Sanyal *et al*. (1997)
Unscheduled DNA synthesis, human liver slices *in vitro*	–	NT	730	Beamand *et al.* (1998)
Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		320 po × 1	Edwards *et al.* (2000)
Micronucleus formation, $B6C3F_1$ mice *in vivo*	–		300 po; 13 w	National Toxicology Program (1993)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, ICR mice *in vivo*	–		130 po × 6[f]	Morris & Ward (1992)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro test, µg/mL; in-vivo test, mg/kg bw/day; inj, injection; po, oral; w, week
[c] Only in the presence of hamster S9
[d] Slightly toxic dose
[e] Mix of strains TA7001-7006
[f] Treatments on days 1–3 and 5–7 of one week

hepatotoxicity. Susceptibility to liver toxicity, in the rat at least, is also associated with extensive biliary excretion.

Coumarin is toxic in the liver of rats and the liver and lung of mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Coumarin is a natural product occurring in the essential oils of a large number of plants, such as cinnamon, cassia, lavender and woodruff. It is used for its fragrance in many personal care products (perfumes, deodorants, soaps) and in tobacco, in household and industrial products to mask unpleasant odours and, in some countries, as a flavouring agent in food and beverages. It has also been used to treat several medical conditions. Exposure to coumarin may occur from its production, its natural presence in many plants and essential oils, and its several industrial, medical and consumer uses.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Coumarin has been adequately tested by oral administration in two experiments in mice and in one experiment in rats. In mice of one strain, it produced increases in lung tumours (adenomas and carcinomas) in both males and females and in hepatocellular adenomas in females. There was no increase in tumour incidences in another strain of mouse. In one study in rats, coumarin produced a low incidence of renal tubule adenomas in males, seen only after step-sectioning of the kidney. Three other studies in rats could not be evaluated.

5.4 Other relevant data

Coumarin is rapidly and extensively absorbed after topical or oral administration to human subjects. It undergoes very extensive metabolism along two major pathways, 7-hydroxylation and ring-opening to *ortho*-hydroxyphenylacetaldehyde. There are numerous minor metabolites, many of which are secondary products from the primary metabolites. The relative extent of these two major pathways is highly variable between species. Ring-opening predominates in rodents, while 7-hydroxylation is particularly evident in humans.

In humans exposed to coumarin for treatment of various clinical conditions, a few cases of hepatotoxicity have been reported. However, a clear relationship between the

dose of coumarin and the hepatotoxic responses observed has not been established. The target organs for coumarin toxicity are primarily the liver in rats and the liver and lung in mice. There are marked species differences in these responses, with the mouse being particularly susceptible to coumarin-induced Clara cell injury. Coumarin is hepatotoxic in rats and mice. Hamsters and gerbils are resistant to acute coumarin-induced hepatotoxicity. *In vitro*, coumarin is toxic in either hepatocytes or liver slices from rats, mice, rabbits and guinea-pigs, whereas monkey and human cells and/or slices appear to be resistant.

No data on reproductive and developmental effects in humans were available. No signs of teratogenicity were observed in mice, rats, rabbits or miniature pigs.

No data were available on the genetic and related effects of coumarin in humans.

Coumarin did not induce micronuclei in mice *in vivo* and was not mutagenic in *Drosophila melanogaster.* It was weakly positive in induction of micronuclei in human cells *in vitro*, but failed to induce unscheduled DNA synthesis in human liver cells *in vitro.* Coumarin induced sister chromatid exchanges without metabolic activation and chromosomal aberrations with metabolic activation, but not micronuclei or gene mutations in mammalian cells *in vitro*. It was mutagenic in only two out of 11 *Salmonella typhimurium* strains tested, with metabolic activation.

Coumarin was antimutagenic in various assays, but also had co-mutagenic properties.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of coumarin were available.

There is *limited evidence* in experimental animals for the carcinogenicity of coumarin.

Overall evaluation

Coumarin is *not classifiable as to its carcinogenicity to humans (Group 3).*

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