

## CINNAMYL ANTHRANILATE

This substance was considered by previous working groups, in June 1977 (IARC, 1978), October 1982 (IARC, 1983) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

### 1. Exposure Data

#### 1.1 Chemical and physical data

##### 1.1.1 Nomenclature

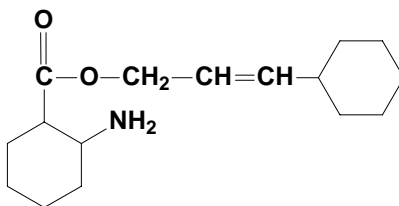
*Chem. Abstr. Serv. Reg. No.:* 87-29-6

*Chem. Abstr. Name:* 3-Phenyl-2-propen-1-ol, 2-aminobenzoate

*IUPAC Systematic Name:* Anthranilic acid, cinnamyl ester

*Synonyms:* Cinnamyl alcohol anthranilate; 3-phenyl-2-propenyl 2-aminobenzoate; 3-phenyl-2-propenyl anthranilate

##### 1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_{15}NO_2$

Relative molecular mass: 253.30

##### 1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Crystalline solid (Budavari, 1996); brownish powder with a balsamic, fruity odour (Burdock, 1995)

- (b) *Boiling-point*: 332 °C (National Toxicology Program, 1991)
- (c) *Melting-point*: 61–61.5 °C (Budavari, 1996)
- (d) *Density*: 1.18 g/cm<sup>3</sup> at 15.5 °C (Burdock, 1995)
- (e) *Solubility*: Very slightly soluble in water (< 1 mg/mL at 17 °C); very soluble in acetone and dimethyl sulfoxide; soluble in chloroform, diethyl ether and ethanol (National Toxicology Program, 1991)
- (f) *Stability*: Sensitive to oxidation in air, photooxidation and hydrolysis (National Toxicology Program, 1991)
- (g) *Conversion factor*<sup>1</sup>: mg/m<sup>3</sup> = 10.36 × ppm

#### 1.1.4 *Technical products and impurities*

Cinnamyl anthranilate is not known to be currently commercially available.

#### 1.1.5 *Analysis*

Cinnamyl anthranilate can be assayed by a method based on ester hydrolysis. Bulk samples of food-grade cinnamyl anthranilate have been analysed for purity by thin-layer chromatography and high-performance liquid chromatography. A method has been described for determining the content of this compound in food products by steam distillation followed by paper chromatography and examination under ultra-violet light; it has a limit of detection of 1 µg (IARC, 1983).

## 1.2 **Production**

Cinnamyl anthranilate can be synthesized by esterification of anthranilic acid with cinnamyl alcohol (Burdock, 1995). Annual production in the United States in the 1970 was in the range of a few hundred kg (IARC, 1983). It has not been commercially available, except for research purposes, since 1985 (Lucas *et al.*, 1999; Food and Drug Administration, 1999).

## 1.3 **Use**

Cinnamyl anthranilate was used for nearly 50 years at very low levels as a synthetic flavouring and fragrance agent. It was used as a flavouring agent to impart a grape or cherry flavour in non-alcoholic beverages, ice cream and ices, sweets, baked goods, gelatins and puddings and chewing gum. It has been used as a fragrance ingredient in various cosmetic products including soaps, detergents, creams and lotions and as a perfume ingredient in orange blossom, neroli, cologne and other blends (Opdyke, 1975; IARC, 1983; National Toxicology Program, 1991; Budavari, 1996).

---

<sup>1</sup> Calculated from: mg/m<sup>3</sup> = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

## **1.4 Occurrence**

### *1.4.1 Natural occurrence*

Cinnamyl anthranilate is not known to occur as a natural product.

### *1.4.2 Occupational exposure*

No data were available to the Working Group.

### *1.4.3 Environmental occurrence*

No data were available to the Working Group.

## **1.5 Regulations and guidelines**

Although cinnamyl anthranilate was classified in the past as a Generally Recognized As Safe (GRAS) substance by the Food and Drug Administration, its use in human food has been prohibited in the United States since 1985. The Food and Agriculture Organization and the World Health Organization have recommended that cinnamyl anthranilate not be used in food (FAO/WHO, 1981; National Toxicology Program, 1991; 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 male and 50 female B6C3F<sub>1</sub> mice, six weeks of age, were fed diets containing 0, 15 or 30 g/kg cinnamyl anthranilate (96% pure; at least five unidentified impurities were found by thin-layer chromatography and two by high-performance liquid chromatography) for 103 weeks. The doses were selected on the basis of a sub-chronic experiment. Survival rates at the end of the study (105–107 weeks) in the control, low-dose and high-dose groups were 88, 82 and 80% in males and 78, 82 and 74% in females. Dose-related reductions in mean body weight were noted in both males and females. A significant dose-related increase ( $p < 0.001$ , Cochran-Armitage

trend test) in the incidence of hepatocellular carcinomas was found in both males and females: females—1/50, 8/49 ( $p = 0.014$ ) and 14/49 ( $p < 0.001$ ) in the control, low- and high-dose groups, respectively; males—6/48, 7/50 and 12/47 [ $p = 0.047$ ]. There was a significant dose-related increase in the incidence of liver-cell carcinomas and adenomas combined in males and females ( $p < 0.001$ , Cochran-Armitage test for trend) (males—14/48 controls, 30/50 low-dose, 37/47 high-dose; females—3/50 controls, 20/49 low-dose, 33/49 high-dose). A few metastases were seen in the lungs of high-dose females (National Cancer Institute, 1980).

### 3.1.2 Rat

Two groups of 50 male and 50 female Fischer 344 rats, seven weeks of age, were fed diets containing 0, 15 or 30 g/kg cinnamyl anthranilate (same sample as used above) for 103 weeks. The dietary levels were selected on the basis of a subchronic study. Surviving animals were killed at 105–107 weeks, at which time 64, 80 and 80% of the males and 78, 88 and 92% of the females were still alive in the control, low- and high-dose groups, respectively. Dose-related reductions in mean body weight were noted in both males and females. There was an increased incidence of mineralization and inflammation of the kidneys of treated rats. A non-statistically significant increase in the incidence of renal tubule tumours was observed in high-dose male rats (4/49; two adenocarcinomas and two adenomas); there was also a non-statistically significant increase in the incidence of acinar-cell pancreatic tumours (3/45: one carcinoma and two adenomas). No such tumour was observed in the matched controls. [The Working Group noted that the historical control incidence in male rats for renal tubule tumours was 0.37% and that that for acinar-cell pancreatic tumours was 0.28%] (National Cancer Institute, 1980).

## 3.2 Intraperitoneal administration

*Mouse:* Cinnamyl anthranilate was tested in a lung adenoma screening assay. In the first series, groups of 15 male and 15 female A/He mice, six to eight weeks of age, were given thrice weekly intraperitoneal injections of cinnamyl anthranilate [purity unspecified] dissolved in tricapylin, as 24 doses of 500 mg/kg bw (the maximal tolerated dose, as found in subchronic experiments) or 100 mg/kg bw (total doses, 12 and 2.4 g/kg bw, respectively). A control group of 25 females (but no control males) received intraperitoneal injections of the vehicle according to the same schedule. All animals were killed 24 weeks after the first injection and lung tumours were found in 10/15 high-dose females, 11/13 high-dose males, 7/15 low-dose females, 10/15 low-dose males and 10/22 control females. The numbers of tumours per mouse were  $0.59 \pm 0.13$  in control females,  $1.50 \pm 0.37$  in low-dose females,  $2.14 \pm 0.70$  ( $p < 0.05$ ) in high-dose females,  $1.13 \pm 0.29$  in low dose males and  $2.69 \pm 0.75$  ( $p < 0.05$ ) in high-dose males (Stoner *et al.*, 1973).

In the second mouse lung adenoma assay, cinnamyl anthranilate was administered to 15 females and 15 males under similar experimental conditions, except that redistilled tricapylin was used as the vehicle. The control group consisted of 80 females and 80 males. The numbers of tumours per mouse were increased at the high dose, being  $0.85 \pm 0.23$  ( $p < 0.01$ ) in high-dose females,  $1.40 \pm 0.36$  ( $p < 0.001$ ) in high-dose males,  $0.54 \pm 0.15$  ( $p < 0.05$ ) in low-dose females and  $0.47 \pm 0.12$  in low-dose males compared with  $0.20 \pm 0.02$  in control females and  $0.24 \pm 0.03$  in control males (Stoner *et al.*, 1973).

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

##### 4.1 Absorption, distribution, metabolism and excretion

###### 4.1.1 Humans

Five human volunteers took a single oral dose of 250 mg cinnamyl anthranilate in water and no unchanged compound was detected in the 0–24-h urine, using analytical methods able to detect 0.04% of the dose (Keyhanfar & Caldwell, 1996).

###### 4.1.2 Experimental systems

Bronaugh *et al.* (1985) determined the percutaneous absorption of cinnamyl anthranilate *in vivo* in rhesus monkeys and *in vitro* through human skin. Absorption through the shaved abdominal skin of four adult female rhesus monkeys after a 24-h application of  $4 \mu\text{g}/\text{cm}^2$  was  $26.1 \pm 2.3\%$  of the dose, which rose to  $39.0 \pm 2.8\%$  when the site of application was covered with an occlusive dressing. Penetration through excised full-thickness human abdominal skin was determined using diffusion cells with a non-ionic surfactant (6% oleth 20, a polyethylene glycol 20 ether) as the receptor fluid. After application of  $4 \mu\text{g}/\text{cm}^2$  cinnamyl anthranilate,  $24.0 \pm 5.1\%$  ( $n = 8$ ) of the dose was absorbed within 48 h. When the surface of the skin was occluded, the penetration rose to  $53.3 \pm 6.7\%$  of the dose ( $n = 7$ ).

The metabolism of cinnamyl anthranilate in rats and mice has been studied (Keyhanfar & Caldwell, 1996). Male Fischer 344 rats and male CD-1 mice were given  $250 \text{ mg}/\text{kg}$  bw [ $3\text{-}^{14}\text{C}$ ] cinnamyl anthranilate by intraperitoneal injection. The majority of the administered  $^{14}\text{C}$  was excreted in the 0–24-h urine (70% of the dose in rats and 78% in mice). A further 10% (rat) and 6% (mouse) was recovered in the 24–72-h urine with 10% (rat) and 7% (mouse) in the 0–72-h faeces. In the rat, the major urinary metabolite was hippuric acid (95% of urinary  $^{14}\text{C}$ ), together with much smaller amounts of benzoic acid. However, in mice, the urine contained relatively less hippuric acid (~80%) and more benzoic acid (16% of urinary  $^{14}\text{C}$ ), together with 2.2% of the dose as unchanged cinnamyl anthranilate.

In further studies, the effect of intraperitoneal dose size upon the fate of cinnamyl anthranilate in mice was examined over a range of 5–250 mg/kg bw. No intact ester was found in the urine after 5 mg/kg bw, but at 50 mg/kg bw, 3.1% of the dose was excreted as cinnamyl anthranilate and, at 250 mg/kg, the percentage was 2.2% (Keyhanfar & Caldwell, 1996).

In an earlier study, Caldwell *et al.* (1985) gave a single oral dose of 500 mg/kg bw to B6C3F<sub>1</sub> mice and found 0.3–0.4% of the dose as unchanged cinnamyl anthranilate in the 0–24-h urine, accompanied by 17% as anthranilic acid and 35% as hippuric acid. Cinnamyl anthranilate was detected in the plasma, rapidly declining from peak levels seen at 0.5 h after dosing. The peak levels were some 3.5 times higher in males than in females.

The influence of dose size was also examined in male and female B6C3F<sub>1</sub> mice given 0, 10, 100, 1000, 5000, 15 000 or 30 000 ppm (mg/kg diet) cinnamyl anthranilate in the diet for four days (Caldwell *et al.*, 1985). The urinary excretion of cinnamyl anthranilate, hippuric acid and anthranilic acid within 24-h after removal of the test diet rose with increasing cinnamyl anthranilate dose. Cinnamyl anthranilate was detected in increasing quantities in the urine of male mice at 1000 ppm and above. In females, it was only seen at 5000 ppm and above and the levels were two- to nine-fold lower.

## 4.2 Toxic effects

### 4.2.1 Humans

No data were available to the Working Group.

### 4.2.2 Experimental systems

Dietary consumption (30 g/kg diet) of 11 g/kg bw per day by female B6C3F<sub>1</sub> mice and 3 g/kg bw per day by female Fischer 344 rats for up to 13 weeks was not associated with lethality, although body weight gain was diminished (Lake *et al.*, 1997). Similar dietary administration (15 or 30 g/kg diet) did not affect survival in studies of eight weeks' and two years' duration (National Toxicology Program, 1980).

Cinnamyl anthranilate was shown to be a hepatic enzyme inducer in mice by Caldwell *et al.* (1985). Male and female B6C3F<sub>1</sub> mice were fed diets containing 0, 10, 100, 1000, 5000, 15 000 or 30 000 ppm (mg/kg diet) cinnamyl anthranilate for 19 days. The relative liver weight (% of body weight) and hepatic microsomal cytochrome P450 content increased in a dose-dependent fashion, significant above 1000 ppm: this was more evident in males than females, but the maximum response (approximately twofold) was the same in both sexes. Although the P450 marker activities, aniline hydroxylase and *para*-nitroanisole *O*-demethylase, in the 9000 × *g* supernatant were unaltered by cinnamyl anthranilate administration, SDS-PAGE examination of the liver microsomes showed marked induction of a P450 isozyme of 53.1 kDa. The dose threshold for increased liver weight and microsomal enzyme induction was the same, and correlated

with that for excretion of unchanged cinnamyl anthranilate noted in the shorter (four-day) experiment (Section 4.1.2).

The hepatic effects of cinnamyl anthranilate were evaluated in male CD1 mice and male Fischer 344 rats treated by intraperitoneal injection for three consecutive days (Viswalingam & Caldwell, 1997). At doses of 100 and 1000 mg/kg bw per day, relative liver weights of mice increased by 22% and 50%, respectively, 24 h after the final dose and peroxisomal (cyanide-insensitive) palmitoyl-coenzyme A (CoA) oxidation activity increased fivefold at both levels. Microsomal lauric acid 11- and 12-hydroxylase activity (CYP4A) was increased 15-fold at 100 mg/kg bw per day and 17-fold at 1000 mg/kg bw per day. Limited evaluation indicated that cinnamyl anthranilate increased the size and number of peroxisomes in electron micrographs of hepatocytes of treated mice. In rats, relative liver weights and peroxisomal palmitoyl-CoA oxidation activity were significantly increased only at 1000 mg/kg bw per day (22% and twofold, respectively).

In a separate experiment, groups of male CD1 mice were given intraperitoneal injections of 0–200 mg/kg bw cinnamyl anthranilate daily for three days. At doses of 20 mg/kg bw and above, there were dose-dependent increases in relative liver weight, total cytochrome P450, and cyanide-insensitive palmitoyl-CoA oxidation. The hepatic effects of cinnamyl anthranilate are apparently due to the intact ester, since neither its expected metabolites alone nor an equimolar mixture of the hydrolysis products, cinnamyl alcohol and anthranilic acid, had a significant effect on the weight or marker enzyme content of mouse liver (Viswalingam & Caldwell, 1997).

Lake *et al.* (1997) have confirmed and extended these findings. Female B6C3F<sub>1</sub> mice and female Fischer 344 rats were fed diets containing 0–30 mg/kg diet (11 030 mg/kg bw per day in mice, 2700 mg/kg bw per day in rats) cinnamyl anthranilate for one, four or 13 weeks. In mice, feeding cinnamyl anthranilate at  $\geq 0.15\%$  (439 mg/kg bw per day) resulted in dose-dependent increases in relative liver weight and hepatic peroxisome proliferation, as demonstrated by the induction of peroxisomal (cyanide-insensitive) palmitoyl-CoA oxidation (see Table 1). Microsomal lauric acid 12-hydroxylase activity (CYP4A) was similarly increased. Hepatocellular replication (measured as nuclear 5-bromo-2'-deoxyuridine [BrdU] labelling) was increased during week 1 of cinnamyl anthranilate treatment at  $\geq 0.15\%$  in the diet (439 mg/kg bw per day) and continued to be elevated during weeks 4 and 13 at  $\geq 0.30\%$  (883 mg/kg bw per day). In contrast to mice, rats fed cinnamyl anthranilate had much smaller increases in relative liver weight and peroxisomal palmitoyl-CoA oxidation, even at the higher dietary concentrations. This apparent difference in magnitude of response was not accounted for by rate of intake (see Table 1). In rats, there was no induction of microsomal lauric acid 12-hydroxylase activity. While BrdU labelling was increased during week 1 of administration in rats, this response was not sustained during weeks 4 or 13, and the magnitude of response during week 1 was again lower in rats.

**Table 1. Comparison of responses in liver of female mice and rats following four weeks of cinnamyl anthranilate treatment**

Diet (%)	Intake (mg/kg bw per day)		Relative liver weight (% increase over controls)		Peroxisomal palmitoyl-CoA oxidation (increase over controls)		Microsomal lauric acid hydroxylase (increase over controls)			
	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse		Rat	
							11-position	12-position	11-position	12-position
0.03	87	NE	NC	NE	NC	NE	NC	NE	NE	NE
0.15	439	159	30	NC	3-fold	NC	3-fold	NE	NC	NC
0.30	883	321	40	NC	4-fold	NC	4-fold	6-fold	NC	NC
0.75	2140	763	60	10	7-fold	2-fold	8-fold	8-fold	2-fold	NC
1.50 <sup>a</sup>	4640	1370	80	15	10-fold	3-fold	12-fold	11-fold	3-fold	NC
3.00 <sup>a</sup>	11 030	2700	100	25	15-fold	6-fold	16-fold	13-fold	6-fold	NC

Adapted from Lake *et al.* (1997)

<sup>a</sup> Dietary levels administered to mice and rats in a carcinogenesis bioassay and associated with increased incidence of liver tumours in mice, but not in rats (National Toxicology Program, 1980).

NC, not different from controls; NE, not evaluated



### 4.3 Reproductive and developmental effects

No data were available to the Working Group.

### 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 2 for references)

Cinnamyl anthranilate was not mutagenic to *Salmonella typhimurium* when tested in the presence or absence of rat liver S9 fraction. It has been reported to give positive results for mutagenic activity in a bioluminescence assay in *Photobacterium phosphoreum* with exogenous activation. It was not active in mutation experiments with *Drosophila* and did not cause sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells. It gave negative or equivocal results for the induction of sister chromatid exchanges in Syrian hamster embryo cells. It did not induce unscheduled DNA synthesis in rat hepatocytes in an in-vivo/in-vitro assay. It has been shown to enhance cell transformation of Syrian hamster embryo cells by SA7 virus. Cinnamyl anthranilate is reported to be active in the mouse lymphoma mutation assay.

### 4.5 Mechanistic considerations

There is a marked species difference in the hepatic effects of cinnamyl anthranilate between mice (both CD-1 and B6C3F<sub>1</sub>) and Fischer 344 rats. In mice, this compound has the characteristic biochemical and morphological effects of a potent peroxisome proliferator, increasing liver weight, fatty acid oxidation, CYP4A isozymes and replicative DNA synthesis (Caldwell *et al.*, 1985; Lake *et al.*, 1997; Viswalingam & Caldwell, 1997). In rats, these effects are much less evident. The species-specificity of peroxisome proliferation has been attributed to differences in the metabolism of cinnamyl anthranilate between rats and mice (Caldwell, 1992; Keyhanfar & Caldwell, 1996; Viswalingam & Caldwell, 1997). Hepatic effects were seen in mice only (*a*) after administration of the intact ester but not an equimolar mixture of its hydrolysis products and (*b*) at dose levels of cinnamyl anthranilate at which intact cinnamyl anthranilate is excreted in the urine. In contrast, rats, which are relatively resistant to its peroxisome-proliferating effect, do not excrete cinnamyl anthranilate in the urine at any dose level.

Some general considerations about the role of peroxisome proliferation as a mechanism of carcinogenicity are presented in the General Remarks section of this volume. Studies of this mechanism are reviewed fully in Section 4.5 of the monograph on di(2-ethylhexyl) phthalate in this volume.

**Table 2. Genetic and related effects of cinnamyl anthranilate**

Test system	Result <sup>a</sup>		Dose <sup>b</sup> (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3600 µg/plate	Wild <i>et al.</i> (1983)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate	Tennant <i>et al.</i> (1987)
<i>Photobacterium phosphoreum</i> , bioluminescence assay, reverse mutation	–	+	NR	Elmore & Fitzgerald (1990)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	–	–	1267 in feed	Wild <i>et al.</i> (1983)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	–	–	2000 ppm, inj <sup>c</sup> ; 5000 in feed	Foureman <i>et al.</i> (1994)
Mutation, mouse lymphoma L5178Y cells, <i>Tk</i> locus <i>in vitro</i>	NT ?	+	10	Tennant <i>et al.</i> (1987)
Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	–	–	30 µg/mL	Gulati <i>et al.</i> (1989)
Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	–	–	40 µg/mL	Gulati <i>et al.</i> (1989)
Cell transformation, Syrian hamster embryo cells <i>in vitro</i>	?	NT	50 µg/mL	Tu <i>et al.</i> (1986)
Cell transformation, C3H/10T½ mouse cells	–	NT	40 µg/mL	Dunkel <i>et al.</i> (1988)
Cell transformation, SA7 virus/Syrian hamster embryo cells	+	NT	20.3	Hatch <i>et al.</i> (1986)
Unscheduled DNA synthesis, male Fischer 344 rats <i>in vivo</i>	–	–	1000 po × 1	Mirsalis <i>et al.</i> (1989)
Micronucleus formation, male B6C3F <sub>1</sub> mouse bone-marrow cells <i>in vivo</i>	?	–	1000 ip × 3	Shelby <i>et al.</i> (1993)
Micronucleus formation, male and female NMRI mouse bone-marrow cells <i>in vivo</i>	–	–	2533 ip × 1	Wild <i>et al.</i> (1983)

<sup>a</sup> +, positive; –, negative; ?, inconclusive; NT, not tested

<sup>b</sup> LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NR, not reported; inj, injection; ip, intraperitoneal

<sup>c</sup> Injection volume not reported

The weight of evidence for cinnamyl anthranilate and for other rodent peroxisome proliferators in general, demonstrates that they do not act as direct DNA-damaging agents.

Chronic administration of peroxisome proliferators to rodents results in sustained oxidative stress due to overproduction of peroxisomal hydrogen peroxide. The induction of peroxisomal fatty acid  $\beta$ -oxidation by cinnamyl anthranilate *in vivo* under bioassay conditions (Lake *et al.*, 1997) supports this hypothesis. Other data on the induction of oxidative stress are not available for cinnamyl anthranilate.

Similarly, the modulation of hepatocellular proliferation by peroxisome proliferators has been implicated in the mechanism of carcinogenesis. This can theoretically result in increased levels of mutation by increasing the frequency of replicative DNA synthesis as well as increasing the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is likely to be involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that cinnamyl anthranilate causes acute and sustained levels of hepatocellular proliferation under bioassay conditions which resulted in liver tumours in mice. Interestingly, the magnitude and duration of hepatocellular proliferation were limited in rats, which did not respond with liver tumours in the bioassay (Lake *et al.*, 1997).

Marked species differences in hepatic peroxisome proliferation have been reported (Ashby *et al.*, 1994; IARC, 1995; Lake, 1995a,b; Cattley *et al.*, 1998). No study has yet compared the responsiveness of human versus rodent livers *in vivo* or hepatocytes *in vitro* to cinnamyl anthranilate; however, a growing body of evidence concerning the molecular basis of peroxisome proliferation indicates that human livers and hepatocytes would be refractory to induction of peroxisome proliferation by cinnamyl anthranilate (Doull *et al.*, 1999).

In summary:

1. The only standard genotoxicity assay in which cinnamyl anthranilate is active is the mouse lymphoma mutation assay.
2. Cinnamyl anthranilate produces liver tumours in mice.
3. Under conditions of the bioassays, cinnamyl anthranilate induced peroxisome proliferation and cell replication in the liver that are characteristic of a peroxisome proliferator in mice and, to a limited extent, in rats.
4. The species difference in peroxisome proliferation in response to cinnamyl anthranilate is associated with a species difference in its metabolism: the compound is completely hydrolysed by rats but not mice.
5. Rodent peroxisome proliferators exercise their pleiotropic effects due to activation of PPAR $\alpha$ . This process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.
6. The absence of a significant response of human liver to induction of peroxisome proliferation and hepatocellular proliferation is explained by several aspects of PPAR $\alpha$ -mediated regulation of gene expression.

7. Hepatic peroxisome proliferation has not been evaluated in studies of human subjects or systems treated with cinnamyl anthranilate. However, interspecies comparisons with other peroxisome proliferators, along with the role of PPAR $\alpha$  in this response, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by cinnamyl anthranilate. This conclusion is further supported by the failure to detect intact cinnamyl anthranilate in the urine of human volunteers given large single doses.
8. Overall, these findings suggest that the increased incidence of liver tumours in mice treated with cinnamyl anthranilate results from a mechanism that is not expected to operate in humans, although studies of human systems have not been performed with this compound.

## **5. Summary of Data Reported and Evaluation**

### **5.1 Exposure data**

Cinnamyl anthranilate was used as a synthetic flavouring and fragrance agent. It has not been commercially available since 1985. No information was available on its occurrence in the workplace or in the environment.

### **5.2 Human carcinogenicity data**

No data were available to the Working Group.

### **5.3 Animal carcinogenicity data**

Cinnamyl anthranilate was tested for carcinogenicity in one experiment in mice and in one experiment in rats by oral administration in the diet. In mice, a dose-related increase in the incidence of hepatocellular tumours was found, but there was no increased incidence of tumours in rats. In a mouse lung tumour bioassay, an increased multiplicity of lung tumours was found.

### **5.4 Other relevant data**

Cinnamyl anthranilate is metabolized by hydrolysis to anthranilic acid and cinnamyl alcohol, which is oxidized to benzoic acid. In mice, but not in rats or humans, the hydrolysis is saturated at high doses, leading to excretion of unchanged cinnamyl anthranilate in the urine.

Cinnamyl anthranilate has the characteristic effects of a peroxisome proliferator on mouse liver, increasing the activity of peroxisomal fatty acid-metabolizing enzymes and microsomal CYP4A and increasing hepatocellular proliferation. These effects are mediated by the intact ester, and were not seen after administration of the hydrolysis products, cinnamyl alcohol and anthranilic acid. The corresponding effects on rat liver were very much weaker. No relevant data from humans were available.

The only standard genotoxicity assay in which cinnamyl anthranilate was active was the mouse lymphoma mutation assay.

### 5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of cinnamyl anthranilate were available.

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

#### Overall evaluation

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

## 6. References

- Ashby, J., Brady, A., Elcombe, C.R., Elliott, B.M., Ishmael, J., Odum, J., Tugwood, J.D., Kettle, S. & Purchase, I.F. (1994) Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. exp. Toxicol.*, **13** (Suppl. 2), S1–S117
- Bronaugh, R.L., Steward, R.F., Wester, R.C., Bucks, D., Mailbach, H.I. & Anderson, J. (1985) Comparison of percutaneous absorption of fragrances by humans and monkeys. *Food chem. Toxicol.*, **23**, 111–114
- Budavari, S., ed. (1996) *The Merck Index*, 12th Ed., Whitehouse Station, NJ, Merck & Co., pp. 387–388
- Burdock, G.A., ed. (1995) *Fenaroli's Handbook of Flavor Ingredients*, 3rd Ed., Vol. II, Boca Raton, FL, CRC Press, p. 116
- Caldwell, J. (1992) Problems and opportunities in toxicity testing arising from species differences in xenobiotic metabolism. *Toxicol. Lett.*, **64/65**, 651–659
- Caldwell, J., Anthony, A., Cotgreave, I.A., Sangster, S.A., Sutton, J.D., Bernard, B.K. & Ford, R.A. (1985) Influence of dose and sex on the disposition and hepatic effects of cinnamyl anthranilate in the B6C3F1 mouse. *Food chem. Toxicol.*, **23**, 559–566
- Cattley, R.C., DeLuca, J., Elcombe, C., Fenner-Crisp, P., Lake, B.G., Marsman, D.S., Pastoor, T.A., Popp, J.A., Robinson, D.E., Schwetz, B., Tugwood, J. & Wahli, W. (1998) Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul. Toxicol. Pharmacol.*, **27**, 47–60

- Doull, J., Cattley, R., Elcombe, C., Lake, B.G., Swenberg, J., Wilkinson, C., Williams, G. & van Gemert, M. (1999) A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new US EPA Risk Assessment Guidelines. *Regul. Toxicol. Pharmacol.*, **29**, 327–357
- Dunkel, V.C., Schechtman, L.M., Tu, A.S., Sivak, A., Lubet, R.A. & Cameron, T.P. (1988) Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. *Environ. mol. Mutag.*, **12**, 21–31
- Elmore, E. & Fitzgerald, M.P. (1990) Evaluation of the bioluminescence assays as screens for genotoxic chemicals. *Prog. clin. biol. Res.*, **340D**, 379–387
- FAO/WHO (1981) *Toxicological Evaluation of Certain Food Additives* (WHO Food Add. Ser. No. 16), Geneva, International Programme on Chemical Safety, pp. 70–73
- Food and Drug Administration (1999) Food and drugs. *US Code Fed. Regul.*, **Title 21**, Part 189, Subpart B, Section 189.113, p. 549
- Foureman, P., Mason, J.M., Valencia, R. & Zimmering, S. (1994) Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ. mol. Mutag.*, **23**, 208–227
- Gulati, D.K., Witt, K., Anderson, B., Zeiger, E. & Shelby, M.D. (1989) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. III. Results with 27 chemicals. *Environ. mol. Mutag.*, **13**, 133–193
- Hatch, G.G., Anderson, T.M., Lubet, R.A., Kouri, R.E., Putman, D.L., Cameron, J.W., Nims, R.W., Most, B., Spalding, J.W., Tennant, R.W. & Schechtman, L.M. (1986) Chemical enhancement of SA7 virus transformation of hamster embryo cells: evaluation by interlaboratory testing of diverse chemicals. *Environ. Mutag.*, **8**, 515–531
- IARC (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 16, *Some Aromatic Amines and Related Nitro Compounds—Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals*, Lyon, IARCPress, pp. 287–291
- IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 31, *Some Food Additives, Feed Additives and Naturally Occurring Substances*, Lyon, IARCPress, pp. 133–139
- IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Update of IARC Monographs Volumes 1 to 42*, Lyon, IARCPress, p. 60
- IARC (1995) *Peroxisome Proliferation and its Role in Carcinogenesis* (IARC Technical Report No. 24), Lyon, IARCPress
- Keyhanfar, F. & Caldwell, J. (1996) Factors affecting the metabolism of cinnamyl anthranilate in the rat and mouse. *Food chem. Toxicol.*, **34**, 241–249
- Lake, B.G. (1995a) Peroxisome proliferation: current mechanisms relating to nongenotoxic carcinogenesis. *Toxicol. Lett.*, **82/83**, 673–681
- Lake, B.G. (1995b) Mechanisms of hepatocarcinogenicity of peroxisome-proliferating drugs and chemicals. *Ann. Rev. Pharmacol. Toxicol.*, **35**, 483–507
- Lake, B.G., Price R.J., Cunninghame, M.E. & Walters D.G. (1997) Comparison of the effects of cinnamyl anthranilate on hepatic peroxisome proliferation and cell replication in the rat and mouse. *Fundam. appl. Toxicol.*, **39**, 60–66
- Lucas, C.D., Putnam, J.M. & Hallaghan, J.B. (1999) *Flavor and Extract Manufacturers' Association of the United States: 1995 Poundage and Technical Effects Update Survey*, Washington DC, Flavor and Extract Manufacturers' Association of the United States, p. 61

- Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P. & Spalding, J.W. (1989) Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. *Environ. mol. Mutag.*, **14**, 155–164
- National Cancer Institute (1980) *Bioassay of Cinnamyl Anthranilate for Possible Carcinogenicity* (Tech. Rep. Ser. No. 196; DHEW Publ. No. (NIH) 80-1752, No. (NTP) 80-10), Washington DC, Government Printing Office
- National Toxicology Program (1991) *NTP Chemical Repository Report: Cinnamyl Anthranilate*, Research Triangle Park, NC
- Opdyke, D.L.J. (1975) Fragrance raw materials monographs: cinnamaldehyde anthranilate. *Food Cosmet. Toxicol.*, **13**, (Suppl. 2), 751–752
- Shelby, M.D., Erexson, G.L., Hook, G.J. & Tice, R.R. (1993) Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. *Environ. mol. Mutag.*, **21**, 160–179
- Stoner, G.D., Shimin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K. & Gori, G.B. (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. *Cancer Res.*, **33**, 3069–3085
- Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B. & Minor, R. (1987) Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science*, **236**, 933–941
- Tu, A., Hallowell, W., Pallotta, S., Sivak, A., Lubet, R.A., Curren, R.D., Avery, M.D., Jones, C., Sedita, B.A., Huberman, E., Tennant, R., Spalding, J. & Kouri, R.E. (1986) An inter-laboratory comparison of transformation in Syrian hamster embryo cells with model and coded chemicals. *Environ. Mutag.*, **8**, 77–98
- Viswalingam, A. & Caldwell, J. (1997) Cinnamyl anthranilate causes co-induction of hepatic microsomal and peroxisomal enzymes in mouse but not rat. *Toxicol. appl. Pharmacol.*, **142**, 338–347
- Wild, D., King, M.-T., Gocke, E. & Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the *Salmonella*/microsome, Basc and micronucleus tests. *Food chem. Toxicol.*, **21**, 707–719