

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Statement for Council of Europe: The carcinogenicity of coumarin with particular reference to the possible mechanism of hepatocarcinogenicity in the rat

Background to request

1. The Committees on Mutagenicity (COM) and Carcinogenicity (COC) of Chemicals in Food, Consumer Products and the Environment are independent advisory committees which report to the Chief Medical Officer. Their terms of reference include, at the request of UK Government Departments, assessing and advising on the mutagenic and carcinogenic risks to man of substances which are used or proposed to be used as food additives or which may be present in food as contaminants.

2. The Joint Food Safety and Standards Group of the Department of Health and the Ministry of Agriculture Fisheries and Food has requested advice on the interpretation of the available carcinogenicity and mutagenicity data on coumarin and whether the substance should be regarded as a genotoxic carcinogen. The toxicology data are under consideration by the Council of Europe's Committee of Experts on Flavouring Substances (CEFS) as part of its review of flavouring source materials and this committee, of which the UK is a member, has asked for advice from national experts on the genotoxic potential of coumarin.

Introduction

3. Coumarin (2H-1-benzopyran-2-one) is present as a major constituent in plants such as tonka beans and as a minor constituent in certain edible fruits such as strawberries, cherries and apricots.^{1,2} It also occurs in natural flavouring source materials such as cinnamon and is also used in fragrances. Limits on coumarin levels in food as a result of its presence in natural flavouring sources are set in Directive 88/388/EEC.³ These limits, which were recommended by the EU Scientific Committee for Food (SCF), were based on those previously set by the CEFS in its review of natural flavouring source materials.⁴ An extensive review of the toxicology of coumarin was subsequently carried out by the SCF in 1994 which concluded that coumarin was carcinogenic in rats via the oral route and possibly in mice. The SCF considered that the genotoxicity of coumarin could not be ruled out and that the general permitted level in foods and beverages should be reduced from 2 mg/kg to the currently achievable limit of detection (0.5 mg/kg) and action be taken to reduce levels permitted in other specific products.² The CEFS is currently considering coumarin again as part of a further toxicological review of natural flavouring source materials and it is for current discussions at this committee that the opinions of the expert committees, the COM and COC, have been sought.

4. The COM and COC have primarily considered the adequacy of the available

mutagenicity data but have also commented on the significance of the data for human health and the possible mechanisms for carcinogenicity documented in rodents.

Metabolism

5. There is a substantial amount of data on the pharmacokinetics and metabolism of coumarin which has recently been reviewed.⁵ Coumarin is rapidly absorbed from the gastrointestinal tract and undergoes extensive metabolism in the liver. In most species, including humans, coumarin is predominantly excreted as metabolites in the urine whereas significant biliary excretion and enterohepatic recirculation takes place in the rat. The major inter-species differences in metabolism have been known for some years. Essentially, the major pathway in the rat involve hydroxylation at position 3 of the pyrone ring giving rise via a number of metabolic steps, to *o*-hydroxyphenyl acetic acid which is excreted in the urine. In baboons and humans, the predominant route involves hydroxylation of the phenyl ring giving rise to 7'-hydroxycoumarin which is then excreted in the urine in a conjugated form, mainly as a glucuronide. However, the metabolism of coumarin in humans has been shown to be very variable and complex. In addition the rate and extent of 7'-hydroxylation of coumarin depends on a variety of factors including diet, smoking, liver disease, impaired renal function and treatment with certain medicines. There is also some evidence for genetic polymorphism in humans in the metabolism of coumarin.⁵

6. A number of research groups have examined whether there is any correlation between species differences in coumarin metabolism and the documented inter-species differences in coumarin hepatotoxicity.⁶⁻⁹ Initial studies in rats suggested that 3'-hydroxylation might lead to the formation of a reactive intermediate (3,4-epoxide) which might be important in the mechanism of hepatotoxicity.⁶⁻⁸ However there is no satisfactory correlation between 3'-hydroxylation in experimental animals and the occurrence of hepatotoxicity.⁹ Thus 3'-hydroxylation occurs in the Syrian hamster, but this species is insensitive to the hepatotoxic effects of coumarin.

7. The relevant metabolic pathways and specific P450 isozymes involved in the hepatotoxicity and carcinogenicity in rodents have not been elucidated and thus no definite conclusions on the significance of the available metabolism data for human health assessment can be drawn at present. It is therefore not possible to discount the relevance for human health of the findings from carcinogenicity bioassays on the basis of metabolism data, particularly in view of the evidence for variation in coumarin metabolism in humans.

Mutagenicity

8. The COM assessed the available mutagenicity data and reached the following conclusions.

- i) Coumarin has been fairly extensively investigated for its genotoxicity *in vitro*.¹⁰⁻²⁴ Positive results were obtained in the Salmonella assay with strain TA100 in the presence of rat or hamster S9.²¹ Coumarin has also been shown to produce chromosome aberrations in a metaphase analysis study in CHO cells* in the presence of rat S9.²¹ There is also some evidence for the induction of SCE in CHO cells.¹²¹ Negative results were recently obtained in an assay

for UDS using human liver slices.¹⁵ The *in vitro* data indicate that coumarin has mutagenic potential.

- ii) Negative results were obtained when coumarin was investigated in the sex-linked recessive lethal assay in *Drosophila melanogaster*; the compound was administered both in the diet or injection.²¹
- iii) Negative results were obtained in inadequately conducted bone marrow micronucleus tests using non-standard protocols.^{25,26} Negative results were also obtained in a peripheral blood micronucleus assay in mice following sub-chronic (90 day) exposure as part of an NTP study.²¹ However, this study was limited to examination of normochromatic erythrocytes of peripheral blood only and no investigation of the bone marrow had been undertaken.
- iv) The Committee concluded that coumarin was clearly genotoxic *in vitro* and that further data were necessary to provide reassurance that it was not genotoxic *in vivo* (rodent bone marrow micronucleus and rat liver UDS assays conducted to OECD guideline standards).

[* Abbreviations used by COM. CHO cells = Chinese Hamster Ovary cells, SCE = Sister Chromatid Exchange, UDS = Unscheduled DNA Synthesis, NTP = National Toxicology Programme of USA, OECD = Organisation for Economic Cooperation and Development]

9. The COM asked for advice from the COC on the mechanisms of tumorigenesis particularly with respect to the occurrence of cholangiocarcinomas documented in a carcinogenicity bioassay in Sprague-Dawley rats.^{27,28}

Carcinogenicity

10. The COC considered the available carcinogenicity bioassays. A brief summary of the critical data is given below:

Rat

- i) Coumarin was fed to groups of 50 male and 50 female Sprague-Dawley rats in the diet at 333, 1000, 2000, 3000 or 5000 ppm for 104 weeks (males) and 110 weeks (females). Rats receiving 333, 1000 or 2000 ppm were also exposed to these dietary levels during gestation and lactation. The maximum tolerated dose (MTD) was exceeded at dietary levels of 2000 ppm and above which may limit the value of these results for human health assessment. A statistically significant increase in cholangiocarcinoma was documented in males and females fed 5000 ppm (approximately 230 mg/kg bw/day in males and 280 mg/kg bw/day in females). A single cholangiocarcinoma was documented in a male rat fed 3000 ppm. Statistically significant increases in hepatocellular adenomas and hepatocellular carcinomas were also documented at 5000 ppm. Increased incidence of non-neoplastic liver pathology was documented in male and female animals at 5000 ppm and, to a lesser extent, at 3000 ppm which included cholangiofibrosis, cystic bile ducts and

hepatocellular degeneration.^{27,28}

- ii) Coumarin was fed to groups of 40 male and 40 female Sprague-Dawley rats in the diet at 200, 600, 1800 or 5400 ppm for 137 weeks. Satellite groups of 10-40 animals of each sex per dose level were also included in the study to investigate clinical chemical effects and to conduct interim histology. The MTD was clearly exceeded at the top dose level which equated to dose levels of approximately 300-340 mg/kg bw/day. An increase in the incidence of malignant hepatoma was reported in male and female rats at the top dose level.^{29,30} Many of the animals fed 5400 ppm were reported to have proliferative cholangiofibrosis of the liver.^{29,30} [We understand that a number of these lesions have been subsequently described as cholangiocarcinomas in a peer review of slides].
- iii) Groups of 50 male and 50 female F344 rats were given oral doses of 25, 50 or 100 mg/kg bw/day of coumarin in corn oil for 5 days per week for up to 103 weeks as part of the NTP testing programme. A significant increase in mortality due to nephropathy was seen in males and females at 50 and 100 mg/kg bw/day and only 2 animals in these dose groups survived to termination. An increased incidence of renal tubule adenomas was noted at all dose levels in males, but the increase was only statistically significant at the mid-dose level. A slight increase in renal tubule adenomas was also reported in high dose females. There was no evidence for an increase in the incidence of liver or bile duct tumours in this study although an increase in the incidence of bile duct hyperplasia, and coagulative necrosis/fibrosis and cytologic alterations of liver parenchyma were reported in males and females at the top dose level.²¹

Mouse

- iv) Coumarin was fed to groups of 52 male and 52 female CD1 mice in the diet at 300, 1000 or 3000 ppm for 137 weeks. The intake of coumarin at the top dose level equated to approximately 270-280 mg/kg bw/day. Body weight gain was reduced by 18% and 10% at 3000 ppm and 1000 ppm respectively during the first 52 weeks only. Food utilisation was marginally reduced in the top dose male animals. These data suggest that the MTD was achieved in this study (and may have been exceeded at the high dose level). There was no evidence reported of a treatment related carcinogenic effect in this study.²⁸
- v) Groups of 50 male and 50 female B6C3F₁ mice were given oral doses of 50, 100 or 200 mg/kg bw/day of coumarin in corn oil for 5 days per week for up to 103 weeks as part of the NTP testing programme. Body weights were approximately 3-10% below controls in top dose males between week 10-81. Mean body weights in females were 3-18% below controls during weeks 11-49 and about 12% lower at the end of the study. These data suggest that the MTD was achieved in this study. Statistically significant increases in the incidence of alveolar/bronchiolar adenomas were reported in top dose male and female animals and alveolar/bronchiolar carcinomas in top dose females. A

statistically significant increase in the incidence of hepatocellular adenoma was reported in low and mid dose level females. A marginal increase in the incidence of forestomach papillomas was reported in low dose male and female animals.²¹

11. The COC agreed that the finding of lung and forestomach tumours in mice probably represented high dose species-specific effects and were unlikely to be of significance for human health assessment following exposure to low levels of coumarin in the diet. Thus most emphasis was placed on the finding of liver, bile duct and kidney tumours in rats. Members agreed that the renal adenomas seen in F344 rats may have been induced by coumarin. The finding of significant non-neoplastic pathology including nephropathy in male and female animals and renal tubule hyperplasia in male animals suggested that non-genotoxic mechanisms may have played a role in the aetiology of these tumours. Members agreed that the hepatocellular carcinomas and cholangiocarcinomas reported in Sprague-Dawley rats were induced by coumarin, but occurred at dose levels which exceeded the MTD. It was noted that treatment related non-neoplastic pathology including bile duct cysts and hyperplasia and degenerative changes and necrosis of the liver parenchyma, had been documented in Sprague-Dawley rats given the high dose level, suggesting non-genotoxic mechanisms may have played a role.

12. However, Members were aware that cholangiocarcinomas had been reported in rats in bioassays where both genotoxic and non-genotoxic chemical carcinogens had been tested and agreed it was not possible to draw any definite conclusions regarding the mechanism of coumarin carcinogenicity in the absence of adequate mutagenicity data on coumarin. It was noted that hepatotoxicity had been reported at a very low incidence (ca 0.2-0.4%) among patients treated with coumarin in respect of cancer or chronic infections.^{31,32} The hepatotoxicity in humans may involve an idiosyncratic reaction, but it is not possible to discount the involvement of toxic metabolites. Thus these data strengthened the need for adequate *in vivo* mutagenicity data on coumarin.

Conclusion

13. The COM and COC agreed that there were insufficient data to draw any definite conclusions regarding the mechanism of coumarin-induced carcinogenicity in the rat. It was agreed that in view of the evidence for mutagenic effects *in vitro*, the first step should be the provision of adequate *in vivo* mutagenicity data. In this respect adequate rodent bone marrow micronucleus and rat liver UDS assays conducted to the current OECD guidelines were required.

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