SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of curcumin (E 100) as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of curcumin (E 100). Curcumin has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF). In 2004 JECFA allocated an ADI of 0-3 mg/kg bw/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel considered that the indications provided by the positive results for curcumin in several *in vitro* and *in vivo* tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded, and that the available *in vivo* genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity. The Panel noted that all statistically significant effects noted by NTP in a long-term carcinogenicity study in rats and mice refer to benign neoplastic lesions (adenomas) and that the incidences for malignant neoplastic lesions (carcinomas) did not reach statistical significance. The Panel also noted that the effects observed were not dose-dependent, were in line with historical control values and not consistent across sexes and/or species. The Panel agreed with JECFA that curcumin is not carcinogenic. The Panel also concluded that this eliminates the concerns over genotoxicity. The Panel concluded that the present database supports an ADI of 3 mg/kg bw/day based on the NOAEL of 250-320 mg/kg bw/day from the reproductive toxicity study for a decreased body weight gain in the F2 generation observed at the highest dose level, and an uncertainty factor of 100. The Panel concluded that at the maximum levels of use, intake estimates for 1- to 10-year old children at the mean and the high percentile (95^{th}) are above the ADI in some European countries. The Panel noted that intake of curcumin from the normal diet amounts to less than 7% of the ADI of 3 mg/kg bw/day.

KEY WORDS

Curcumin, E 100, CAS Registry Number 458-37-7, 33171-16-3, 33171-05-0, Turmeric Yellow, Kurkum, INS no. 100(i), CI Natural Yellow 3, Diferoylmethane, EINECS number 207-280-5, Colour index number 75300.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific Opinion re-evaluating the safety of curcumin (E 100) when used as a food colouring substance.

Curcumin (E 100) is a dicinnamoylmethane dye authorised as a food additive in the EU and was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974, 1978, 1980, 1982, 1987, 1990, 1992, 1995, 2000, 2002 and 2004, and the EU Scientific Committee on Food (SCF) in 1975. The SCF concluded in 1975 that an Acceptable Daily Intake (ADI) could not be established, but that curcumin was nevertheless acceptable for use in food. In 2004 JECFA allocated an ADI of 0-3 mg/kg bw/day.

Curcumin consists of three principal colouring components. It consists essentially of curcumins i.e. the colouring principle (1E, 6E)-1, 7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1, 6-diene-3, 5-dione and its desmethoxy- and bis-desmethoxy-derivatives.

Regarding the kinetics of curcumin, in animal studies it appeared that curcumin is rapidly metabolised and excreted (mainly via faeces). Studies in humans also reveal that it is unlikely that substantial concentrations of curcumin occur in the body after ingestion at doses up to 12 000 mg/person, equivalent to 200 mg/kg bw for a 60 kg person.

Curcumin was studied for genotoxicity in a battery of short-term assays of genetic toxicity. The compound was not active in the following assays: *Salmonella*/microsome test using strains TA98 and TA100, sister chromatid exchange (SCE) using hamster lung fibroblasts and human embryo fibroblasts. Neither curcumin nor commercial turmeric oleoresin (containing 17.5% of curcumin) were active in the *Salmonella*/mammalian microsome test using TA1535, TA100 and TA98 strains. Curcumin was reported not to induce chromosome damage in Chinese hamster ovary cells (CHO) cells *in vitro*. The results of several other genotoxicity studies on turmeric or curcumin revealed no mutagenic activity in bacteria treated with turmeric preparations containing up to 85% curcumin.

Positive results were reported in the rec assay (*Bacillus subtilis*) and for chromosomal aberrations in hamster lung fibroblasts. A 79-85% purity preparation induced chromosomal aberrations and SCEs *in vitro*. Blasiak et al. (1999), provided evidence that curcumin induced DNA damage (measured as DNA-strand breaks in the Comet assay) in human lymphocytes and gastric mucosa cells *in vitro* when present in the low micromolar range (10-50 μM), and furthermore that curcumin works in an additive fashion with hexavalent chromium, a well known mutagen and carcinogen. Antunes et al. (1999), substantiated this finding in that curcumin was found to induce DNA damage in CHO cells at a concentration of 10 μM, and potentiated the effect of doxorubicin, a known free radical generator.

In an *in vivo* study in mice injected ***i.p.*** with curcumin of unknown purity there was some evidence of SCE induction at low frequency at concentrations above 25 mg/kg bw, while in rats fed curcumin of unknown purity there was equivocal evidence for the induction of chromosomal aberrations.

Weanling Swiss albino mice fed control diets or diets containing 0.5% turmeric (curcumin content unknown) or 0.015% curcumin of unknown purity (equivalent to 20 mg/kg bw/day) for 12 weeks were used in several genetic toxicity tests. Groups of eight females given curcumin or turmeric exhibited no effect in the micronucleus test. Groups of five males and five females given turmeric or curcumin showed no cytogenetic effect on the bone marrow chromosomes. Similarly no effect of the substances was noted in a dominant lethal study in which 15 male and 45 female mice were exposed to the test diets.

In 1996, JECFA concluded that no genotoxicity studies with high purity curcumin were available and that in limited studies with curcumin preparations of up to 85% purity, or of unknown purity, no

mutagenic activity was seen in bacteria and only equivocal activity was seen in assays for the induction of chromosomal aberrations. JECFA concluded that there was no evidence to show that curcumin was genotoxic. In the last evaluation by JECFA from 2004 no new studies were evaluated.

The TemaNord evaluation (2002) stated that no *in vitro* genotoxicity studies with high-purity curcumin are available. Employing curcumin preparations of a purity of up to 85%, no mutagenicity has been observed in the Ames assay or in assays studying chromosomal aberrations. Furthermore, curcumin potentiated the effect of doxorubicin, a known free radical generator.

Several new *in vitro* genotoxicity studies are available since these previous evaluations. Curcumin induced DNA damage (measured in the Comet assay), damage in both mitochondrial and nuclear DNA in HepG2 cells and a small but significant increase in micronuclei in HepG2 cells.

Also a new *in vivo* study in rats is available. Curcumin spice caused a statistically significant dose-dependent increase in the number of micronucleated polychromatic erythrocytes (MNPCEs) and in the frequencies of total chromosomal aberrations over the control in male rats which received a suspension of curcumin spice (not further specified) corresponding to 0.5, 5, 10, 25 and 50 mg/kg bw in 1 ml distilled water orally, daily, for four weeks.

In Long-Evans Cinnamon (LEC) rats, exposure to 0.5% curcumin (95% purity) in the diet enhanced etheno-DNA adduct formation 9- to 25-fold in nuclear DNA and 3- to 4-fold in mitochondrial DNA. LEC rats are a model for human Wilson's disease and develop chronic hepatitis and liver tumours owing to accumulation of copper and induced oxidative stress.

The Panel considered that the indications provided by the positive results for curcumin in several *in vitro* and *in vivo* tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded, and that the available *in vivo* genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity.

In 1993 the National Toxicology Program (NTP) reported the results of long-term studies in which rats and mice were exposed to levels of 0, 2000, 10000 or 50000 mg/kg diet turmeric oleoresin (79-85% curcumin) for 103 weeks. For the rat study the NTP concluded that there was no evidence of carcinogenic activity of turmeric oleoresin in male F344/N rats, but equivocal evidence of carcinogenic activity of turmeric oleoresin in female F344/N rats based on increased incidences of clitoral gland adenomas in the exposed groups.

For mice, the NTP concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin in male B6C3F1 mice based on a marginally increased incidence of hepatocellular adenoma at the 10 000 mg/kg diet level, and the occurrence of carcinomas of the small intestine in the 2000 and 10 000 mg/kg diet groups (although the Panel noted that the incidences were not statistically significant). For female B6C3F1 mice, the NTP concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin based on an increased incidence of hepatocellular adenomas in the 10 000 mg/kg diet group.

The Panel noted that all statistically significant effects noted by the NTP refer to benign neoplastic lesions (adenomas) and that the incidences for malignant neoplastic lesions (carcinomas), including the carcinomas of the small intestine in male mice, did not reach statistical significance. The Panel also noted that the effects observed were not dose-dependent, were in line with historical control values and were not consistent across sexes and/or species. Therefore the Panel agreed with JECFA that curcumin is not carcinogenic. The Panel also concluded that this eliminates the concerns over genotoxicity.

Regarding the reproductive toxicity of curcumin, JECFA concluded on the basis of a multigeneration study in rats, that were fed with curcumin for periods of up to 24 weeks, that the No-Observed-Adverse-Effect Level (NOAEL) was 250–320 mg/kg bw/day, since decreased body-weight gain in the F2 generation was observed at doses equal to 960–1100 mg curcumin/kg bw/day. The current JECFA

ADI of 0-3 mg/kg bw/day is based on this NOAEL of 250-320 mg/kg bw/day with the application of an uncertainty factor of 100.

The Panel considered the decreased body weight gain in the F2 generation observed at the highest dose level an adverse effect and agrees with the NOAEL allocated by JECFA of 250-320 mg/kg bw/day.

The Panel concluded that the present database supports an ADI of 3 mg/kg bw/day, based on the NOAEL of 250-320 mg/kg bw/day from the reproductive toxicity study for a decreased body weight gain in the F2 generation observed at the highest dose level and an uncertainty factor of 100.

The dietary exposure to curcumin was estimated by the Panel based on the maximum permitted levels (MPLs) of use, by applying the Budget method (Tier 1) with the assumptions described in the report of the Scientific Cooperation (SCOOP) Task 4.2. The Panel calculated a theoretical maximum daily exposure of 6.9 mg/kg bw/day for adults and of 11.9 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPLs of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of curcumin listed in Table 3 (Tier 3), as identified by the Panel from the data made available by Industry. For children (1-10 years old), estimates have been calculated from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Sweden, Czech Republic, Greece, Cyprus and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for curcumin exposure estimates.

When considering MPLs (Tier 2), estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and 3.3 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'non alcoholic flavoured drinks'). The mean dietary exposure of European children (aged 1-10 years) ranged from 0.5 to 3.8 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile.

When considering the maximum reported use levels (Tier 3), estimates reported for the UK adult population give a mean dietary exposure to curcumin of 0.8 mg/kg bw/day and 2.0 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'non alcoholic flavoured drinks'). The mean dietary exposure of European children (aged 1-10 years) ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.1 to 7.1 mg/kg bw/day at the 95th percentile.

This exposure assessment does not however take into account the use of turmeric (*Curcuma longa)* as a spice in cooking. The use of turmeric as a spice added to foods and used in home-made recipes was assessed using data from Irish adults (aged 18-64 years) and children (aged 5-12 years). Intakes of curcumin from turmeric as a spice consumed by adults (consumers only, n=66) ranged from a mean intake of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile, while intakes of curcumin based on the intake of curry powder (consumers only, n=91) also ranged from a mean intake of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile. In children, intakes of curcumin from turmeric as a spice (consumers only, n=7) ranged from a mean intake of 0.1 mg/kg bw/day to 0.2 mg/kg bw/day at the 97.5th percentile, while intakes of curcumin based on the intake of curry powder (consumers only, n=21) ranged from a mean intake of curcumin of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile.

The combined exposure to curcumin from naturally occurring curcumin in foods (turmeric as spice and in curry powder) and from its use as a food colour using the anticipated exposure estimates from Tier 3 is estimated to be in the range of 0.7 to 3.6 mg/kg bw/day for children and at 1.0 mg/kg bw/day for adults on average. For the combined exposure at the 95th percentile, the range was estimated from 1.6 to 7.6 mg/kg bw/day for children and at 2.6 mg/kg bw/day for adults.

The Panel concluded that intake from the normal diet amounts to less than 7% of the ADI of 3 mg/kg bw/day, resulting from an average exposure to curcumin of 0.1 mg/kg bw/day from the intake of turmeric and curry powder each for both children and adults.

The purity of curcumin is specified as not less than 90% total colouring matters. The Panel noted that the specifications for curcumin should be updated to define the residual 10%.

The Panel noted that specifications for curcumin according to Commission Directive 2008/128/EC and JECFA differ with regard to the solvents that are allowed for extraction and purification of curcumin and their maximum levels in the material of commerce, and with regard to the maximum allowed lead concentration (which is given as ≤ 10 mg/kg and ≤ 2 mg/kg respectively) and with regard to other metals.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established by EFSA in 2008.

The Panel also noted that the aluminium lake of the colour may lead to an anticipated aluminium exposure of up to 3.84 mg/kg bw/week (in UK pre-school children, for high level consumers of panned and compressed confectionery), which exceeds the Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week, and that therefore specifications for the maximum level of aluminium in the lakes may be required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the framework Directive 89/107/EEC[4] on food additives, the Scientific Committee for Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor the European Food Safety Authority (EFSA).

Directive 89/107/EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives[5] which will apply as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

In accordance with Regulation (EC) No 1333/2008, the Commission should, after consultation with EFSA, set up by 20 January 2010 an evaluation programme for EFSA to re-evaluate the safety of the permitted food additives. That programme will define the needs and the order of priorities according to which the approved food additives are to be examined.

Food colours were among the first additives to be evaluated therefore, many of the evaluations are old. For some of these colours new studies have become available and the results of these studies should be included in the evaluation. Therefore, food colours should be evaluated with priority. The order of priorities for the re-evaluation of the remaining permitted food additives will be set in the Regulation for the re-evaluation program.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to start a systematic re-evaluation of all authorised food additives and to issue scientific opinions on these additives, taking into account that colours as a group should be given the highest priority for the reasons outlined above.

[4] OJ L 40, 11.2.1989, p. 27
[5] OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of curcumin (E 100) when used as a food colouring substance.

Curcumin (E 100) is a dye authorised as a food additive in the EU and previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974, 1978, 1980, 1982, 1987, 1990, 1992, 1995, 2000, 2002 and 2004.

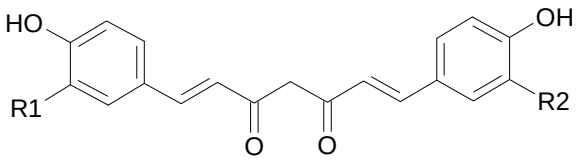
The ANS Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substance

Curcumin (E 100) is a dicinnamoylmethane food dye consisting of three principal colouring components. The product consists essentially of curcumins i.e. the colouring principle (1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, with the molecular formula $C_{21}H_{20}O_6$ and molecular weight of 368.39 g/mol, and its desmethoxy- and bis-desmethoxy-derivatives with molecular formulas $C_{20}H_{18}O_5$ and $C_{19}H_{16}O_4$ and molecular weights of 338.39 and 308.39 g/mol respectively, in varying proportions.

The structural formulas of the three principal colouring components are given in Figure 1.



I.	$C_{21}H_{20}O_6$;	$R_1 = R_2 = OCH_3$	CAS Registry Number 458-37-7
II.	$C_{20}H_{18}O_5$;	$R_1 = OCH_3$, $R_2 = H$	CAS Registry Number 33171-16-3
III.	$C_{19}H_{16}O_4$;	$R_1 = R_2 = H$	CAS Registry Number 33171-05-0

Figure 1: Structural formula of three principal components of curcumin (I, II and III)

Curcumin is an orange-yellow crystalline powder which is water-insoluble but soluble in ethanol.

More than 45 synonyms are mentioned in ChemIDplus (via Internet, 2007). Turmeric Yellow, Kurkum, INS no. 100(i), CI Natural Yellow 3 and diferoylmethane are frequently used synonyms.

2.2. Specifications

Specifications have been defined in Directive 2008/128/EC[6] on purity criteria concerning colours for use in foodstuffs and JECFA (JECFA, 2006) (Table 1).

The product consists essentially of curcumins i.e. the colouring principle 1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione and its desmethoxy- and bis-desmethoxy-derivatives in varying proportions.

The purity is specified as not less than 90% total colouring matters. The Panel noted that the specification should be updated to define the residual 10%.

Table 1: Specifications for curcumin according to Commission Directive 2008/128/EC and JECFA (JECFA, 2006)

Purity	Commission Directive 2008/128/EC	JECFA (2006)
Solvent residues:		
- Ethyl acetate	≤ 50 mg/kg (singly or in combination)	≤ 50 mg/kg*
- Acetone		≤ 30 mg/kg
- *n*-Butanol		-
- Methanol		≤ 50 mg/kg*
- Ethanol		≤ 50 mg/kg*
- Hexane		≤ 25 mg/kg
- Isopropanol	-	≤ 50 mg/kg*
- Dichloromethane	≤ 10 mg/kg	-
Arsenic	≤ 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals	≤ 40 mg/kg	-

* Single or in combination with ethyl acetate, methanol, ethanol and/or isopropanol.

The Panel noted that specifications for curcumin according to Directive 2008/128/EC and JECFA (2006) differ with regard to the solvents that are allowed for extraction and purification of curcumin and their maximum levels in the material of commerce and with regard to the maximum allowed lead concentration (which is given as ≤ 10 mg/kg and ≤ 2 mg/kg respectively) and with regard to other metals.

Minor amounts of oils and resins naturally occurring in turmeric may be present in the final preparation. According to JECFA (2006), only the following solvents may be used in the extraction and purification: acetone, methanol, ethanol, isopropanol, hexane, ethyl acetate. Supercritical carbon dioxide may also be used in the extraction. According to Directive 2008/128/EC only the following solvents may be used in the extraction: ethyl acetate, acetone, carbon dioxide, dichloromethane, *n*-butanol, methanol, ethanol and hexane.

According to EU legislation (Directive 2008/128/EC), the above purity criteria for the pure substance also apply to the raw material from which the aluminium lake is produced. In addition, the aluminium lake should contain no more than 0.5% HCl-insoluble material, and no more than 0.2% ether-extractable material under neutral conditions. There are no additional specification requirements for the aluminium lake.

[6] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

JECFA does not give specifications for aluminium lakes of curcumin, other than reference to the General Specifications for Aluminium Lakes of Colouring Matters (JECFA, 2006). The curcumin used in the production process should comply with the specifications as given above, and the aluminium lake should contain not more than 2% water-soluble chlorides and sulphates calculated as sodium salts, not more than 0.5% HCl-insoluble matter, 0.2% ether-extractable matter, 3 mg arsenic/kg and 5 mg lead/kg. Unreacted aluminium oxide may also be present in the final product (not specified).

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

Curcumin is obtained by solvent extraction of turmeric i.e. the ground rhizomes of natural strains of *Curcuma longa* L. (*Curcuma domestica* Valeton). In order to obtain a concentrated curcumin powder, the extract is purified by crystallisation.

Curcumin may be converted to its corresponding aluminium lake under aqueous conditions by reacting aluminium oxide with the colouring matter. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulphate or aluminium chloride with either sodium carbonate, sodium bicarbonate or aqueous ammonia. Following lake formation, the product is filtered, washed with water and dried (JECFA, 2004). The aluminium lake of curcumin has a quite high ratio of aluminium: pigment (2:1) and is to some extent similar to the more conventional lakes made with synthetic colours (based on single supplier data).

2.4. Methods of analysis in foods

Several methods for the determination of curcumin in foods are described in the literature, of which combinations of high performance liquid chromatography (HPLC) with various detection methods appear to be most generally employed (Krishna et al., 2009).

2.5. Reaction and fate in foods

The principal colouring components of curcumin are relatively stable at acidic pH, but they rapidly decompose at pHs above 7. In a study of alkaline degradation of diferuloylmethane (Tonnesen and Karlsen, 1985), products of decomposition at pH 7-10 were determined by HPLC. Ferulic acid and feruloylmethane are formed initially. Feruloylmethane rapidly forms coloured (mostly yellow to brownish-yellow) condensation products. Degradation products formed by hydrolysis of feruloylmethane are vanillin and acetone and their amount increase with incubation time.

The principal colouring components of curcumin are not particularly stable to light, especially in solutions. After the photo-irradiation of diferuloylmethane, a cyclisation product was detected, as well as decomposition products, such as vanillic acid, vanillin, and ferulic acid (Sasaki et al., 1998). Commercial formulations of curcumin that are designed to minimize the inherent light instability are available.

2.6. Case of need and proposed uses

Authorised uses and use levels of curcumin have been defined in the Directive 94/36/EC[7].

Currently, curcumin is an authorised food colour in the EU, with maximal allowed use levels varying from 20 to 500 mg/kg food according to food type. Curcumin is also allowed in beverages at levels up to 200 mg/l. Table 2 summarises those beverages and foodstuffs that are permitted to contain curcumin up to specified maximum levels set by Directive 94/36/EC.

Information on current maximum use levels was made available to the Panel for several food categories in finished products by the Confederation of the Food and Drink Industries of the EU (CIAA) and NATCOL. These are detailed in Table 2.

Table 2: Maximum Permitted Levels of use of curcumin in beverages and foodstuffs according to the European Parliament and Council Directive 94/36/EC and maximum reported use levels of curcumin in beverages and foodstuffs used for the refined exposure assessment (Annex A)

Beverages	**Maximum Permitted Level** (mg/l)	**Maximum Reported Level** (mg/l)
Non-alcoholic flavoured drinks	100	100[1]
Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100	100[2]
Spirituous beverages	200	–*
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200	200[2]
Foodstuffs	**Maximum Permitted Level** (mg/kg)	**Maximum Reported Level** (mg/kg)
Sausages, patês and terrines	20	2.5[1]
Complete formulae for weight control intended to replace total daily food intake or an individual meal	50	10[1]
Complete formulae and nutritional supplements for use under medical supervision	50	50[2]
Soups	50	35[1]
Savoury snack products and savoury coated nuts	100	75[1]
Flavoured processed cheese Fish paste and crustaceans paste Smoked fish Meat and fish analogues based on vegetable proteins	100	100[2]
Desserts including flavoured milk products Edible ices	150	150[1]
Fine bakery wares Candied fruit and vegetables, Mostarda di frutta Preserves of red fruits	200	200[1]
Extruded or expanded savoury snack products	200	75[1]
Pre-cooked crustaceans	250	250[1]
Confectionery Mustard Fish roe	300	300[1]
Solid food supplements/dietary integrators	300	10[1]
Decorations and coatings	500	500[1]

[7] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 273, 10.9.94, p.13.

Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi		
Margarine, minarine, other fat emulsions, and fats essentially free from water	*Quantum satis*	40[1]
Edible casings Pasturmas (edible external coating)	*Quantum satis*	50[3]
Jam, jellies and marmalades as mentioned in Directive 79/693/EEC and other similar fruit preparations including low calorie products	*Quantum satis*	100[1]
Edible cheese rind	*Quantum satis*	50[3]
Dried potato granules and flakes	*Quantum satis*	500[3]

* No usages reported by industry
[1] Maximum use level or maximum level determined by analysis
[2] Maximum permitted level
[3] Q*uantum satis* data (Annex A)

2.6.1.1. Beverages

In October 2009, the Confederation of the Food and Drink Industries of the EU (CIAA, 2009) reported typical use levels data of 70 mg/l for non-alcoholic flavoured drinks.

The association for Natural Colours (NATCOL, Tennant, 2007a) provided use level data for non alcoholic beverages ranging from 2-100 mg/l.

The CIAA (2009) reported no use of curcumin in spirituous beverages, including products with less than 15% alcohol.

2.6.1.2. Foodstuffs

For specific foodstuffs, the CIAA provided the Panel with the following typical use levels of curcumin per kg of food (CIAA, 2009).

Confectionery products (10-300 mg/kg), decorations and coatings (150-500 mg/kg), fine bakery wares (4-200 mg/kg), edible ices (0.64-150 mg/kg), desserts, including flavoured milk products (1-74 mg/kg), sauces and seasonings (2-500 mg/kg), soups (2.5-33 mg/kg), jams, jellies and marmalades (5-30 mg/kg), extruded or expanded savoury snack products (typical value of 12 mg/kg), complete formulae for weight control (typical value of 10 mg/kg), solid food supplements (typical value of 10 mg/kg), sausages, pates and terrines (typical value of 2.5 mg/kg) and for margarine/minarine (typical value of 10 mg/kg).

The association for Natural Colours (NATCOL) also provided use level data for the following foodstuffs per kg of food: confectionery (5-300 mg/kg), decorations and coatings (10-500 mg/kg), fine bakery wares (1-200 mg/kg), edible ices (2-150 mg/kg), flavoured processed cheese (50-100 mg/kg), desserts, including flavoured milk products (1-150 mg/kg), sauces and seasonings (2-300 mg/kg), smoked fish (2-25 mg/kg), snack products (10-50 mg/kg), edible cheese rind and casings (10-50 mg/kg), jams, jellies and marmalades (5-100 mg/kg), and margarine/minarine (2-40 mg/kg).

In order to refine the exposure assessment for children and adults to food colours, the Panel has defined some rules to identify maximum reported use levels based on maximum actual usage or maximum analytical data. The rules followed in order to deal with *quantum satis* authorisation, with use level data or observed analytical data, for all regulated colours re-evaluated by the Panel, are given in Annex A. Table 2 summarises the maximum reported use levels for curcumin in beverages and

foodstuffs used for the refined exposure assessment. They have been defined by applying the rules reported in Annex A to the data available to EFSA.

2.7. Information on existing authorisations and evaluations

Curcumin is permitted as a food additive in the EU under Directive 94/36/EC.

The Scientific Committee for Food (SCF) evaluated curcumin in 1975 and the Committee established that curcumin (from natural foods) was a colour for which an ADI could not be established. In principle, colours for which an ADI cannot be established would not be acceptable for use in food, but the Committee recognised that exceptions might be made in the case of compounds which are in fact constituents of food and derived from coloured natural foods by purely physical processes such as curcumin. In 1975 the SCF concluded that these substances are acceptable for use in food provided that the quantities ingested do not differ substantially from the amounts likely to be ingested as a result of the normal consumption of the foods in which they occur naturally.

In 1974, the JECFA Committee established a temporary ADI of 0-0.1 mg/kg bw/day for curcumin. This temporary ADI for curcumin was extended at subsequent JECFA meetings (JECFA, 1978, 1980, 1982, 1987, 1990 and 1992). The ADI was based on the No-Observed-Effect Level of 250 mg turmeric/kg bw/day in a long-term study in rats (Truhaut, 1958) in which rats were fed a diet containing commercial turmeric. An uncertainty factor of 100 was applied (temporary ADI for turmeric was 0-2.5 mg/kg bw/day) and curcumin was considered to be present in turmeric at 3% (0.03 × 2.5 mg/kg bw/day = 0.1 mg/kg bw/day). The JECFA requested an adequate long-term feeding carcinogenicity study in a rodent species using an oleoresin of turmeric with a well-defined curcumin content.

In 1995, the JECFA Committee increased the temporary ADI to 0-1 mg/kg bw/day based on the NOAEL of 220 mg/kg bw/day in a carcinogenicity study in mice using an uncertainty factor of 200 (JECFA, 1995). The JECFA Committee indicated that a reproductive toxicity study with curcumin should be submitted for review and that otherwise it was unlikely that the temporary ADI could be further extended. This temporary ADI was extended in 2000 and 2002 (JECFA, 2000 and 2002).

In 2004, the JECFA Committee allocated an ADI of 0-3 mg/kg bw/day based on the NOAEL of 250-320 mg/kg bw/day in the multigeneration study in rats and the application of an uncertainty factor of 100 (JECFA, 2004).

An additional evaluation can be found in a report released by the Nordic Council of Ministers (TemaNord, 2002) who has taken into account the literature published until 2000. The TemaNord evaluation concluded that further studies with regard to the carcinogenicity and the reproductive toxicity of curcumin are warranted.

The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) published a monograph on the traditional medicinal use of the rhizomes of *Curcuma longa* L. (EMA, 2010).

2.8. Exposure

2.8.1. Exposure assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the scientific cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each

successive Tier, this involved a further refinement of intakes. The approach goes from the conservative estimates that form the first Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers (Annex A).

2.8.1.1. Crude estimates (Budget Method)

The dietary exposure to curcumin from the maximum permitted use levels was estimated using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2 (EC, 1998).

In the case of curcumin, the maximum permitted use level in beverages was 200 mg/l (Directive 94/36/EC). The maximum level in solid foods (500 mg/kg) was reported in rather specific food categories (i.e. "salmon substitutes and surimi") that were treated separately as non-representative. Therefore, the second maximum level (300 mg/kg) was used in the Budget method.

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In fact, even though curcumin may be used in a variety of solid foods and beverages that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed foods with the same colour added even considering brand loyalty. This assumes that a typical adult weighing 60 kg consumes daily 1.5 litres of beverage and 375 gram of solid foods containing curcumin.

The theoretical maximum daily exposure for adults would therefore be:

(200 x 0.1 x 0.25) + (300 x 0.025 x 0.25) = 5 + 1.875 = 6.9 mg/kg bw/day.

For children, the level of curcumin considered in beverages was 100 mg/l (after exclusion of alcoholic drinks) and in solid food was 300 mg/kg. As recommended by SCOOP task 4.2 (EC, 1998) for children, it is assumed that 100% of beverages contain the additive. This conclusion was derived from UK data on consumption of soft drinks by children aged less than 5 years, where the 97.5th percentile of consumption was between 70 and 80 ml/kg bw/day.). This assumes that a typical 3 year-old child weighing 15 kg consumes daily 1.5 litres of beverages and 94 g of solid foods containing curcumin.

The overall theoretical maximum daily exposure to curcumin in children would therefore be:

(100 x 0.1 x 1) + (300 x 0.025 x 0.25) = 10 + 1.875 = 11.9 mg/kg bw/day.

It was noted that curcumin may be used *quantum satis* in a number of food categories, namely dried potato granules and flakes, edible cheese rind and casings, jams, jellies and marmalades, margarine, minarine and pasturmas. Apart from margarine and jams, jellies and marmalade, these are very specific food categories, which are unlikely to be consumed in high amounts on a daily basis. Therefore it was decided to exclude the potential intake of these food categories from the Budget calculation as they are addressed in the more refined tiers of exposure estimates.

2.8.1.2. Refined estimates

Refined exposure estimates have been performed for Tier 2 using national consumption data and maximum permitted use levels, presented in Table 2, and for Tier 3 using the maximum reported use levels presented in Table 2 for children and adult populations (see Annex A).

For adults, the Panel calculated the exposure based in the UK consumption survey as the UK population is considered to be one of the highest consumers of soft drinks in Europe and also because

detailed individual food consumption data (UK NDNS, 2000-2001) are available from the UNESDA report (Tennant et al., 2006) and the NATCOL report (Tennant, 2007a). The maximum permitted levels (MPL's) of use as specified in the Directive 94/36/EC were used for the Tier 2 approach (Table 2), and the maximum reported use levels were used for the Tier 3 approach (Table 2) (see Annex A). Exposure estimates for children (1-10 years old) have been performed by the Panel based on the EXPOCHI consortium detailed individual food consumption data from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Sweden, Czech Republic, Cyprus, Greece and Germany) for Tier 2 and Tier 3. As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant et al., 2006) and the NATCOL report (Tennant, 2007a).

Table 3 summarises the anticipated exposure of children and adults to curcumin.

Tier 2

In the case of curcumin, when considering MPLs of use, estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and 3.3 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'spirituous beverages'). The main contributor to the total anticipated mean exposure to curcumin (>10%) was non-alcoholic flavoured drinks (46%).

The mean dietary exposure of European children (aged 1-10 years and weighing an average of 15.8-29 kg) considered by the EXPOCHI consortium and UK children ranged from 0.5 to 3.8 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to curcumin (>10% in all countries, these contributions differed per country), were non-alcoholic beverages (13-55%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (12-43%), desserts, including flavoured milk products (12-45%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (11-42%) and margarine, minarine and other fat emulsions (11-18%). Confectionery accounted for 11% in one country and candied fruits and vegetables also accounted for 13% of exposure in one country.

Tier 3

Further data suggest that current use levels of curcumin in some food categories are different than the MPL's. Therefore, it was decided that concentration data made available to the Panel by Industry could be used to refine the estimate of dietary exposure to curcumin (Tier 3).

Estimates reported for the UK adult population give a mean dietary exposure to curcumin of 0.8 mg/kg bw/day and 2.0 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'non alcoholic flavoured drinks'). The main contributors to the total anticipated mean exposure to curcumin (>10%) were non-alcoholic beverages (50%).

When considering the maximum reported use levels from Table 2, the mean dietary exposure of European children (aged 1-10 years and weighing 15.8-29 kg), considered by the EXPOCHI consortium and UK children, ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.1 to 7.1 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to curcumin (>10% in all countries these contributions differed per country), were fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (13-47%), desserts (including flavoured milk products) (13-52%), non-alcoholic beverages (15-57%) and sauces and seasonings (11-45%). Confectionery accounted for 14% in one country.

Table 3: Summary of anticipated exposure to curcumin using Tier 2 and Tier 3 (EC, 2001) in the UK adult population and in children from the UK and the EXPOCHI study

	Adult UK population (>18 years old)	**Children UK & EXPOCHI population** (1-10 years old, 15.8-29 kg body weight
	mg/kg bw/day	mg/kg bw/day
Tier 1. Budget method	6.9	11.9
Tier 2. Maximum Permitted Level		
• Mean exposure	0.9	0.5 – 3.8
• Exposure 95th* or 97.5th percentile**	3.3	1.2 – 7.2
Tier 3. Maximum reported use levels		
• Mean exposure	0.8	0.5 – 3.4
• Exposure 95th* or 97.5th percentile**	2.0	1.1 – 7.1

* For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.

** For UK, estimates are based on the UNESDA report which gives the 97.5th percentile intake from beverages plus *per capita* average from the rest of diet (Tennant, 2006).

This exposure assessment does not take into account the use of turmeric (*Curcuma longa)* as a spice in cooking, and this may contribute to the dietary exposure of curcumin as was observed in the intakes of Irish adults and children. The use of turmeric as a spice added to foods and used in home-made recipes was assessed using data from Irish adults (1379 adults aged 18-64 years) and children (594 children aged 5-12 years) (Harrington et al., 2001; IUNA, 2005). Turmeric was considered as a spice added alone to foods, and also the intake of curry powder was examined, as turmeric is a widespread ingredient in curry powder (approximately 30% depending on the blend). Intakes of turmeric as a spice consumed by adults (consumers only, n=66) ranged from a mean of 0.3 g/day to 0.5 g/day at the 97.5th percentile. Based on 3-4% curcumin content in turmeric (Chattopadhyay et al., 2004), exposure to curcumin from this source is estimated to range from a mean intake of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile. Intakes of curry powder consumed by adults (consumers only, n=91) ranged from a mean of 0.7 g/day to 1.6 g/day at the 97.5th percentile, which equates to a mean intake of curcumin of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile. Intakes of turmeric as a spice consumed by children (consumers only, n=7) ranged from a mean intake of 0.1 g/day to 0.2 g/day at the 97.5th percentile, which in terms of curcumin intakes, equates approximately to a mean intake of 0.1 mg/kg bw/day to 0.2 mg/kg bw/day at the 97.5th percentile. Intakes of curry powder consumed by children (consumers only, n=21) ranged from a mean intake of 0.3 g/day to 0.9 g/day at the 97.5th percentile, which equates to a mean intake of curcumin of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile.

In a report by Tennant (2007b) aluminium intake data are provided for UK pre-school children based on curcumin-containing food consumption figures and using the aluminium curcumin pigment ratio 2:1. Aluminium lakes for curcumin are apparently only used in panned and compressed confectionary and in very few brands (particularly with regard to compressed confectionary in which it is only present in one brand on the EU market). or UK pre-school children, the exposure to aluminium from panned and compressed confectionery was 0.30 and 2.05 mg aluminium/kg bw/week for average consumers and 0.41 and 3.84 mg aluminium/kg bw/week for high level consumers (97.5th percentile), respectively. However, the Panel noted that theses estimates can be considered as highly conservative. This is due to the fact that the consumption of aluminium lakes is assumed to be at the typical maximum level of uses reported by Industry in all panned and compressed confectionary foods where curcumin is authorized as a food colour.

The combined exposure to curcumin from naturally occurring curcumin in foods (turmeric as spice and in curry powder) and from its use as a food colour using the anticipated exposure estimates from tier 3 in Table 3 is estimated to be in the range of 0.7 to 3.6 mg/kg bw/day for children and at 1.0 mg/kg bw/day for adults on average. For the combined exposure at the 95th percentile, the range was estimated from 1.6 to 7.6 mg/kg bw/day for children and at 2.6 mg/kg bw/day for adults.

3. Biological and toxicological data

Curcumin has been previously evaluated by JECFA in 1982, 1987, 1996 and 2004, the EU Scientific Committee for Food (SCF) in 1975 and TemaNord in 2002. The present opinion briefly reports the major studies evaluated in these reports and describes additional newly reported literature data in some more detail.

For the current evaluation, only studies with curcumin or purified turmeric extract in which the curcumin concentration is high and specified are taken into account, since otherwise, the test material in the study is not comparable in composition to curcumin used as a colour additive in food.

For some studies it is indicated that the study was OECD GLP-compliant. Several of the studies used for the JECFA and SCF evaluations were performed in the first half of the 1970s when the first GLP guidelines were implemented. OECD GLP guidelines were not promulgated before 1981. It is unclear whether these reported studies comply with OECD and GLP guidelines.

3.1. Absorption, distribution, metabolism and excretion

The JECFA evaluated several studies on the toxicokinetics of curcumin.

Mice

Curcumin was tested in ADME studies in mice and humans (Tullberg et al., 2004). In the mouse study 20 female and 20 male MF1 mice were dosed by oral gavage at 220 mg/kg bw and 50 male and 50 female adult B6C3F1 mice were dosed at 10 mg/kg bw by oral gavage. In animals dosed at 220 mg/kg bw extremely low plasma concentrations were detected, given the high dose administered. There were statistically significant differences between the sexes ($p<0.05$ by unpaired t-test) suggesting a possible difference in elimination and probably due to more extensive first pass metabolism in males. In the mice dosed at 10 mg/kg bw the results suggested the possibility of non-linear kinetics when compared to the data at the higher dose of 220 mg/kg bw, although the authors also stated that a clear conclusion could not be reached because of the poor precision of the plasma concentration data at the lower dose.

Rats

Five male Sprague-Dawley rats were given by gavage a dose of 1 g/kg of curcumin suspended in arachis oil. Between 67 - 87% of the dose was eliminated in the faeces within 72 hours and excretion was highest in the initial 48 hours. Urinary excretion was negligible. Three hours after gavage, curcumin was detected in the plasma of one of four animals. Biliary concentration of curcumin was 1 µg/ml after 30 minutes and remained stable throughout the experiment. The amount collected in the bile during 3 hours was less than 0.0006% of the dose. After 3 hours, about 0.015% of the administered curcumin had accumulated in the liver, kidneys and body fat. Perfusion of curcumin through the liver resulted in a transitory increase in bile flow; 10% of the dose was excreted in the bile within 3 hours after administration. Of the curcumin excreted in the bile, 49% was in the conjugated form. Because of the poor absorption, rapid metabolism and excretion of curcumin it is unlikely that substantial concentrations of curcumin occur in the body after ingestion (Wahlstrom and Blennow, 1978).

In rats receiving a single oral dose of 0.6 mg curcumin, 89% of the dose was excreted in the faeces and 6% in the urine within 72 hours (Holder et al., 1978). When labelled curcumin was administered to cannulated rats by *i.v.* injection, 85% of the dose was recovered in the bile after 6 hours. Major

metabolites included the glucuronides of tetrahydrocurcumin and hexahydrocurcumin, with dihydroferulic acid and ferulic acid present as minor metabolites (Holder et al., 1978).

Male rats weighing 150-200 g were given by gavage a suspension of 400 mg of curcumin in water containing 0.1% Tween 20. About 40% of the dose was excreted unchanged in the faeces over a 5-day period; excretion tapered off after the first 3 days. The remaining 60% of the curcumin was assumed to have been absorbed. Curcumin was not detected in the urine. However, the influence of curcumin administration was noticed in the increased excretion of conjugated glucuronides and sulphates. Negligible amounts of curcumin were found in the blood, liver and kidney. The authors concluded that curcumin is probably undergoing transformation even as it is being absorbed in the gut (Ravindranath and Chandrasekhara, 1980).

Long-term studies in rats reported discoloration of the fur in curcumin-exposed rats and mice and discoloured faeces in rats receiving 50 000 mg/kg curcumin in their food daily (equal to 2 g/kg bw/day) indicating that significant absorption and bioaccumulation of curcumin occurs at the high doses employed in the studies (NTP, 1993). This is in agreement with the absorption studies of Ravindranath and Chandrasekhara (1982) which indicated that after a single high oral dose of 400 mg/rat (equal to 2 g/kg bw) of [^{3}H]-labelled curcumin, only 60% of the dose was excreted within 12 days after administration. However, at the lower doses of 10 and 80 mg/rat (equal to 0.05 and 0.4 g/kg bw), most of the label was excreted within 72 hours. The percentage of dose absorbed (60-66%) was constant regardless of the dose administered. Only about a third of the excreted radioactivity was present as curcumin (Ravindranath and Chandrasekhara, 1982). It is not clear from the study summary if the tritium label was stably included in the molecule.

In a short-term study on a test material containing 79% curcumin in F344 rats and B6C3F1 mice (Lilja, 1984), it was reported that turmeric (presumably curcumin compound I, CAS No 456-77-7) was detected in blood samples from both species at all dose levels and that blood plasma concentrations increased linearly in a dose-related manner over the dietary concentration range of 0.1-2.5%; at the higher dietary level of 5.0%, plasma levels tended to plateau. The data were claimed to be consistent with earlier reports by Wahlstrom and Blennow (1978) and Holder et al. (1978) in that the substance is poorly absorbed from the gut and is rapidly metabolised and excreted (Lilja, 1984).

Humans

Fifteen patients with advanced colorectal cancer received an extract of Curcuma (18 mg of curcumin and 2 mg of the desmethoxy derivative suspended in 200 mg of essential oils derived from *Curcuma* spp.) daily for up to 4 months. The doses were equivalent to 26, 72, 108, 144 and 180 mg of curcumin, with three patients receiving each dose. Neither curcumin, or its glucuronide or sulphate conjugates, or hexahydrocurcumin or hexahydrocurcuminol were detected in plasma or urine after up to 29 days of treatment.

Curcumin was detected in the faeces of all patients. Curcumin sulphate was also detected in the faeces of one of the patients receiving curcumin at a dose of 180 mg/day, which may have been a result of biotransformation in the gut (Sharma et al., 2001).

Twenty-five patients with conditions indicating a high risk of malignancy were given curcumin (purity, 99.3%) for 3 months. The starting dose was 500 mg/day, which was increased stepwise to 1000, 2000, 4000, 8000 and finally 12 000 mg/day. Pharmacokinetic studies were conducted in patients and in healthy volunteers. Serum concentrations of curcumin peaked at 1–2 hours after administration of 4000–8000 mg curcumin and gradually declined within 12 hours. A half-life was not determined. Curcumin was barely detectable in the serum of patients taking 500–2000 mg of curcumin. No curcumin could be detected in urine. Similar results were obtained in two patients who had taken curcumin for more than one month, indicating that repeated administration did not alter the pharmacokinetic profile of this substance (Cheng et al., 2001).

Curcumin was tested in ADME studies in mice and humans (Tullberg et al., 2004). The kinetic parameters for humans were studied following a dose at 1.0 and 10 mg/kg bw, amounting to the ADI set at that time and ten times that ADI. Plasma samples were collected before and at different time intervals after dosing. Data for four subjects for each sex were presented. At both dose levels curcumin was only rarely found above the limit of quantification and the vast majority of samples contained no detectable curcumin at all, although the analytical method was sufficiently sensitive to measure concentrations in the pg/ml range.

A dose escalation study for a curcumin formulation (containing a minimum of 95% concentration of three curcumins: curcumin, bisdesmethoxycurcumin, and desmethoxycurcumin; dose levels from 500-12 000 mg) was performed with twenty-four healthy human volunteers. No curcumin was detected in the serum of subjects administered 500-8000 mg, which may be due to rapid metabolism. Low levels of curcumin were detected in two subjects administered 10 000 or 12 000 mg (Lao et al., 2006).

In vitro

Studies on the absorption of curcumin carried out with everted rat intestinal sacs indicated that curcumin undergoes transformation during absorption from the intestine (Ravindranath and Chandrasekhara, 1981).

Studies indicated that curcumin was rapidly metabolised when incubated with hepatocytes or microsomal suspensions (Wahlstrom and Blennow, 1978).

3.2. Toxicological data

In the current evaluation, only studies with curcumin or purified turmeric extract in which the curcumin concentration is high were taken into account. The Panel noted that unless otherwise stated, the Panel assumes that the term "curcumin" refers to a mixture of curcumin (CAS No. 458-37-7) and its desmethoxy- and bis-desmethoxy-derivatives (CAS No. 33171-16-3 and 33171-05-0).

3.2.1. Acute oral toxicity

The JECFA evaluated four acute oral toxicity studies. LD_{50} values reported upon oral dosing amounted to 2 g/kg bw (test material not specified)(Srimal and Dhawan, 1973) and >10 g/kg bw (test material estimated to contain about 79% curcumin)(Lilja et al., 1983) for mouse and to 5 g/kg bw (test material not specified)(Wahlstrom and Blennow, 1978) and >10 g/kg bw (test material estimated to contain about 79% curcumin)(Lilja et al., 1983) for rat.

No new acute oral toxicity studies have been published since the previous evaluations.

The Panel noted that the acute toxicity of curcumin is low, but this information is considered of little relevance for its safety evaluation for use as a food colour.

3.2.2. Short-term and subchronic toxicity

Several short-term studies were evaluated by JECFA. TemaNord also refers to the studies of Lilja et al. (1983, 1984), but the Panel assumed that these initial NTP reports were included in the final description of the studies on curcumin by NTP in 1993 (NTP, 1993).

B6C3F1 mice (10 animals/sex/dose), aged 8-9 weeks, received turmeric oleoresin (containing approximately 79-85% curcumin) in the diet at concentrations of 0, 0.1, 0.5, 1.0, 2.5, or 5.0% for 13 weeks. These dietary levels were estimated to deliver average daily doses of 0, 150, 750, 1700, 3850 and 7700 mg/kg bw/day to males and 0, 200, 1000, 1800, 4700 and 9300 mg/kg bw/day to females (NTP, 1993). Food intake and body weight gain were recorded weekly. Urinalysis was performed eight days prior to termination and blood was collected for haematology and clinical chemistry analyses at the end of the study. Gross necropsies were performed on all animals and detailed histological examinations were carried out on the control and 5% dose groups only. No significant differences attributable to treatment were observed in body weight gain, mortality, or histopathology, but a dose-related increase in liver weight occurred in both sexes. Decreases were observed in lung weight that were only statistically significant in males from the two highest dose groups, in thymus weight that was only statistically significant at the 2.5% level, and in kidney weight that was only statistically significant in females from the highest dose group. Haematological changes observed were not dose-related and the values were within normal ranges. Clinical chemistry analyses revealed dose-related increases in cholinesterase and phosphorus, which were significant at the 1% and higher dose levels in males and at the highest two dose levels for cholinesterase or top-dose group only for phosphorus in females. A dose-related decrease in creatinine levels occurred in females at all but the lowest dose level, and in males at the top three dose levels. According to NTP there were no biologically significant treatment related effects on haematological parameters, clinical chemistry or urinalysis. JECFA concluded that the no-effect level with respect to gross and microscopic pathological changes was 9280 and 7700 mg/kg bw/day in females and males, respectively, which were the highest doses tested (NTP, 1993). The Panel noted the conclusions of the NTP and of JECFA and concludes that the effects seen on haematological parameters, in clinical chemistry or urinalysis were not accompanied by histopathological changes, and are therefore toxicologically not relevant. The Panel therefore agrees with JECFA that the NOAEL amounts to 9280 and 7700 mg/kg bw/day in females and males, respectively, the highest doses tested.

A 13-week study was carried out in F344 rats using a protocol and the same dose levels as described above for mice. Groups of 10 male and 10 female F344/Ed rats were fed diets containing turmeric oleoresin (containing approximately 79-85% curcumin) at concentrations of 0, 0.1, 0.5, 1.0, 2.5, or 5.0% for 13 weeks (NTP, 1993). These dietary levels were estimated to amount to average daily doses of 0, 50, 250, 480, 1300 and 2600 mg/kg bw/day for males and 0, 60, 300, 550, 1450 and 2800 mg/kg bw/day for females. No significant differences due to treatment were observed in body weight gain, mortality, or histopathology. There was a dose-related increase in liver weight in both sexes; in females there was also a treatment-related decrease in heart and lung weights. Haematological examination showed a dose-related increase in polymorphonuclear lymphocytes at the 2.5 and 5% dose levels in males, while in females there was a small increase only at the 5% level. In females, erythrocyte counts tended to be lower in a treatment-related manner but not in a consistent dose-related manner; other haematological changes were not dose-related. Clinical chemistry analyses in males revealed a number of changes at the mid- to high-dose levels; SGPT [serum glutamic pyruvic transaminase, currently know as alanine transaminase (ALT)], OCT (ornithine carbamyl transferase), total protein, globulin, urea nitrogen, creatinine, and total bilirubin were lower, while the albumin/globulin ratio, direct bilirubin, and chloride tended to be higher than in control. Decreased SGOT [serum glutamic oxaloacetic transaminase, currently known as aspartate transaminase (AST)] and LDH (lactate dehydrogenase) were observed only at the highest-dose level. In females, decreases in comparison to control were observed in LDH, creatinine, total bilirubin, pH, bicarbonate, and total CO_2, while phosphorus was increased at the two higher-dose levels. Urinalysis of male rats indicated that there was a treatment-related increase in casts and an increase in red blood cells at the top two dose levels. Urine of females showed little or no treatment-related change except for increased uric acid crystals at all dose levels and a slight increase in red and white blood cells at the top-dose levels.

According to NTP, there were no biologically significant treatment-related effects on haematological parameters, clinical chemistry or urinalysis. JECFA concluded that the no-effect level with respect to gross and microscopic pathological changes was 5% in the diet, equal to a time-weighted average of 2760 and 2587 mg/kg bw/day in females and males, respectively, which were the highest dose levels tested (NTP, 1993).

The NTP report described that hyperplasia of the mucosal epithelium was observed in the caecum and colon of male and female rats that received the highest dose level (NTP, 1993). Therefore the Panel derived a NOAEL for this 90-day study equal to 2.5% in the diet, equivalent to 1300 mg/kg bw/day for males and 1450 mg/kg bw /day for females, based on hyperplasia at the highest dose level.

In a study in rats, turmeric at levels of 0.3, 1.0 and 10% and curcumin at levels of 0.1, 0.5, 1.0 and 2.0% were included in a synthetic diet and fed to groups of 10 male Wistar strain albino rats for a period of eight weeks. A dose of 10% of turmeric lowered the food efficiency ratio, probably because of reduced food intake. No effects were seen in the other dosed groups as regards growth, haematological values, total serum protein, albumin, globulin and cholesterol. No mortality was seen and no histopathological changes were observed in the gastrointestinal tract, liver, spleen and kidneys (WHO, 1980).

Groups of ten albino Porter strain rats received oral doses of 50 or 100 mg/kg bw of curcumin administered as a 2% suspension in gum arabic daily for 6 days. At the high dose, gastric erosion was reported: changes in the mucin content were reported to be the cause of the ulceration. Pre-treatment with adrenergic, cholinergic, tryptaminergic and histaminergic receptor antagonists provided partial protection, while metiamide pre-treatment completely prevented the development of the lesions (Gupta et al., 1980).

More recently, a 6-month subchronic toxicity study of curcumins extracted from the powdered dried rhizome of *Curcuma longa* L. was performed in six groups of 15 Wistar rats of each sex (Chavalittumrong et al., 2002). The curcumin content and the nature of the extract were not described. The water control group received 5 ml of water/kg bw/day orally, while tragacanth control group received 5 ml of 0.5% tragacanth suspension/kg bw/day orally. Three treatment groups were given the suspension of curcuminoids powder at doses of 10, 50, and 250 mg/kg bw/day. The fourth treatment group, or the recovery group, also received 250 mg/kg bw/day of curcuminoids for 6 months, but two weeks of no curcuminoid treatment elapsed before the time of sacrifice. The growth rate of male rats receiving curcuminoids at 50 mg/kg bw/day was significantly higher than that of the tragacanth control group. Curcuminoids did not produce any significant dose-related changes of haematological parameters. In the group of male animals receiving 250 mg/kg bw/day curcuminoids, actual and relative liver weights and the level of alkaline phosphatase (ALP) were significantly higher than those of the two controls, but the ALP level was still within a normal range. There appeared to be a higher incidence of mild degree of liver fatty degeneration and adrenocortical fatty degeneration in this group of animals; however, the incidence was not significantly different from that of the two controls. The authors concluded that at higher doses, curcumin may affect the function and morphology of the liver in a reversible manner (Chavalittumrong et al., 2002). The Panel concluded that due to the lack of knowledge on the curcumin content and nature of the extract tested, the study cannot be used to assess the safety of curcumin.

In another study transcriptomics were performed upon 28-day dietary administration of curcumin (purity >98%) at dose levels of 26.1, 84.8, 224,8, 459.7 and 1117.8 mg/kg bw/day to Sprague Dawley rats based on cDNA microarray experiments performed on hepatic RNA (Stierum et al., 2008). Clinical chemistry did not reveal major signs of liver damage associated with administration of curcumin. Curcumin altered the expression of 12 genes. Three out of these were related to peroxisomes (phytanoyl-CoA dioxygenase, enoyl-CoA hydratase; CYP4A3). Increased cyanide insensitive palmitoyl-CoA oxidation was observed. The authors concluded that these data suggest that curcumin is a weak peroxisome proliferator.

3.2.3. Genotoxicity

The JECFA evaluated several genotoxicity studies of curcumin. Those studies performed with curcumin or purified turmeric extract in which the curcumin concentration is high and specified are summarised.

Curcumin was studied in a battery of *in vitro* assays for genetic toxicity. The compound was not active in the following assays: *Salmonella*/microsome test using strains TA98 and TA100, sister chromatid exchange (SCE) using hamster lung fibroblasts and human embryo fibroblasts. Positive results were reported in the rec assay (*Bacillus subtilis*) and for chromosomal aberrations in hamster lung fibroblasts (Kawachi et al., 1980).

Neither curcumin nor commercial turmeric oleoresin (containing 17.5% of curcumin) at concentrations of 1.28, 6.4, 32.0 and 160 µg/plate were active in the *Salmonella* test using strains TA1535, TA100 and TA98 with and without metabolic activation (Jensen, 1982).

Curcumin was reported not to induce chromosome damage in Chinese hamster ovary cells *in vitro* (Au and Hsu, 1979).

Weanling Swiss albino mice fed control diets or diets containing 0.5% turmeric (curcumin content unknown) or 0.015% curcumin of unknown purity (equivalent to 20 mg/kg bw) for 12 weeks were used for several genetic toxicity tests (Vijayalaxmi, 1980). Groups of eight females given curcumin or turmeric exhibited no effect in the micronucleus test. Groups of five males and five females given turmeric or curcumin showed no cytogenetic effect on the bone marrow chromosomes. Similarly no effect of the substances was noted in a dominant lethal study in which 15 male and 45 female mice were exposed to the test diets (Vijayalaxmi, 1980).

In 1996, JECFA concluded that no genotoxicity studies with high purity curcumin were available for review and that in limited studies with curcumin preparations of up to 85% purity, or of unknown purity, no mutagenic activity was seen in bacteria and only equivocal activity was seen in assays for the induction of chromosomal aberrations. The Committee concluded that there was no evidence to show that curcumin was genotoxic. In the last evaluation by JECFA (2004), no new studies were evaluated.

NTP concluded based on studies performed (NTP, 1993) that turmeric oleoresin (major component 79-85% curcumin compound I, CAS No 458-37-7) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without exogenous metabolic activation (S9). In an *in vivo* study in mice injected *i.p.* with curcumin of unknown purity there was some evidence of SCE induction at low frequency at concentrations above 25 mg/kg bw (NTP, 1993). Curcumin induced small but significant increases in sister chromatid exchanges (SCE) and chromosomal aberrations in cultured Chinese hamster ovary cells. The positive response in the sister chromatid exchange test occurred in the presence of S9, whereas the aberrations response occurred without S9.

In another study on rats fed curcumin of unknown purity there was equivocal evidence for the induction of chromosomal aberrations (Giri et al., 1990).Two new studies evaluated by TemaNord indicate that curcumin may exert genotoxicity *in vitro,* and also in more recent literature, positive results were found for curcumin (or curcumin spice) in *in vitro* and *in vivo* genotoxicity studies, as described below.

Blasiak et al. (1999a, 1999b) provided evidence that curcumin when present in the low micromolar range (10-50 µM) induced DNA damage (measured as DNA-strand breaks in the Comet assay) in human lymphocytes and gastric mucosa cells *in vitro* and furthermore that curcumin works in an additive fashion with hexavalent chromium, a well known mutagen and carcinogen. Antunes et al. (1999) substantiated this finding in that curcumin was found to induce DNA damage in Chinese hamster ovary cells (CHO) cells at a concentration of 10 µM and potentiated the effect of doxorubicin, a known free radical generator.

Several new *in vitro* genotoxicity studies are available since these previous evaluations.

In Long-Evans Cinnamon (LEC) rats, exposure to 0.5% curcumin (95% purity) in the diet enhanced etheno-DNA adduct formation in nuclear and mitochondrial DNA (Nair et al., 2005) The study reports that LEC rats, a model for human Wilson's disease, develop chronic hepatitis and liver tumours owing to accumulation of copper and induced oxidative stress. Lipid peroxidation (LPO) induced etheno-DNA adducts in nuclear- and mitochondrial-DNA along with apoptosis was measured in LEC rat liver. Levels of etheno-DNA adducts (1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine) increased with age reaching a peak at 8 and 12 weeks in nuclear and mitochondrial DNA, respectively. Apoptosis was assessed by TUNEL +ve cells in liver sections. CD95L RNA expression was also measured by *in situ* hybridization in the same sections. The highest nuclear DNA adduct levels coincided with a reduced apoptotic rate at 8 weeks. Mitochondrial-DNA adducts peaked at 12 weeks that coincided with the highest apoptotic rate, suggesting a link of etheno-DNA adducts in mitochondrial DNA to apoptosis. The DNA damage in liver was further enhanced and sustained by 0.5% curcumin (95% purity) in the diet. Treatment for 2 weeks elevated etheno-DNA adducts 9- to 25-fold in nuclear DNA and 3- to 4-fold in mitochondrial- DNA. The authors indicate that their results confirm the reported *in vitro* DNA damaging potential of curcumin in the presence of copper ions by reactive oxygen species.

Two studies in HepG2 cells are available. Curcumin induced DNA damage (measured in the Comet assay) at concentrations ranging between 2.5-40 µM in HepG2 cells. Furthermore, curcumin induced damage in both the mitochondrial and the nuclear DNA in HepG2 cells, according to studies using quantitative polymerase chain reaction and immunocytochemical staining for 8-hydroxydeoxyguanosine (Cao et al., 2006). Also, curcumin induced a small but significant increase in micronuclei in HepG2 cells at concentrations of 8 and 16 µg/ml, but not at 2 and 4 µg/ml (Cao et al., 2007).

A study reported by Mendonca et al. (2009) aimed to investigate the possible cytotoxicity and genotoxicity/antigenotoxic effects of curcumin in PC12 cells exposed to cisplatin. Cell viability and genotoxicity/antigenotoxicity were evaluated by the MTT assay and micronucleus test, respectively. PC12 cells were treated with different concentrations of cisplatin and curcumin (0.5 - 128 µg/ml). Analysis of the results showed that high concentrations of curcumin were cytotoxic and increased micronuclei frequency compared to the control group. In the associated treatments, at all three concentrations evaluated, curcumin significantly reduced the total frequency of micronuclei induced by cisplatin.

The aim of a study reported by Urbina-Cano et al. (2006) was to evaluate the *in vitro* effect of curcumin in the presence of increasing concentrations of copper to induce DNA damage in murine leukocytes by the Comet assay. Balb-C mouse lymphocytes were exposed to 50 µM curcumin and various concentrations of copper (10, 100 and 200 µM). Cellular DNA damage was detected by means of the alkaline Comet assay. The authors indicated that the results show that 50 µM curcumin in the presence of 100-200 µM copper induced DNA damage in murine lymphocytes. Curcumin did not inhibit the oxidative DNA damage caused by 50 µM H_2O_2 in mouse lymphocytes. Moreover, 50 µM curcumin alone was capable of inducing DNA strand breaks under the tested conditions.

Also a new *in vivo* study in rats is available. Male rats (10 animals/dose) received distilled water by gastric intubation (negative controls), a single *i.p.* injection with 25 mg/kg cyclophosphamide (positive control) or a suspension of curcumin spice (not further specified) corresponding to 0.5, 5, 10, 25 and 50 mg/kg bw in 1 ml distilled water orally, daily, for four weeks. Curcumin spice caused a statistically significant dose-dependent increase in the number of micronucleated polychromatic erythrocytes (MNPCEs) and in the frequencies of total chromosomal aberrations over the control (El-Makawy and Sharaf, 2006). The Panel noted that the curcumin tested was not adequately specified.

The Panel considered that the indications provided by the positive results for curcumin in several *in vitro* and *in vivo* tests for genotoxicity, especially those detecting chromosomal aberrations and DNA

adducts should not be disregarded, and that the available *in vivo* genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

The JECFA evaluated chronic toxicity/carcinogenicity studies in mice and in rats, also referred to in the TemaNord (2002) report.

Mice

B6C3F1 mice (60 animals/sex/dose) were fed *ad libitum* diets containing 0, 2000, 10 000 or 50 000 mg/kg diet turmeric oleoresin (major component 79-85% curcumin compound I, CAS No 458-37-7) for 103 weeks, equal to daily doses of 0, 220/320, 1520/1620 or 6000/8400 mg turmeric oleoresin/kg bw, in males and females, respectively. Dose levels were based on the results of a previous 13-week study.

Mice were housed one per cage and observed twice daily, 7 days/week. Individual animal weights were recorded weekly for the first 13 weeks, then once every four weeks thereafter; food consumption was monitored once every four weeks. An interim sacrifice of 9 or 10 randomly selected mice/group was conducted at 15 months, which included complete gross and microscopic evaluations, assay of a standard set of haematology and clinical chemistry parameters and weights of seven selected organs. At termination, a complete necropsy was performed on all animals, including both gross and microscopic evaluations.

Survival rates were unaffected by dietary turmeric. For male mice, the survival ranged from 74%-86% and for female mice from 68%-84%. At dietary levels of turmeric oleoresin of 10 000 mg/kg (females only) and 50 000 mg/kg diet (males and females), final group mean body weights were significantly lower than controls: however, food consumption in these groups of mice was the same in relation to controls. At 15 months, absolute and relative liver weights were elevated in mice of both sexes fed 10000 and 50 000 mg/kg but returned to control levels at terminal sacrifice. No significant differences in haematological and clinical chemistry parameters were reported, although at 15 months, ALP levels were elevated in males and females receiving the mid- and high- doses. In female mice, turmeric oleoresin was also associated with thyroid gland follicular cell hyperplasia. Table 4 summarises significant results from the statistical analyses of primary tumour data which were presented in the original report. Under the conditions of the study these data showed a marginal increase of neoplasms in mice which was not considered to be treatment-related (NTP, 1993). For male B6C3F1 mice, the NTP concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin based on a marginally increased incidence of hepatocellular adenoma at the 10 000 mg/kg diet level, and the occurrence of small intestine carcinomas in the 2000 and 10 000 mg/kg diet groups. For female B6C3F1 mice, the NTP concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin based on an increased incidence of hepatocellular adenomas in the 10 000 mg/kg diet group (NTP, 1993).

Table 3: Incidence of primary tumours in individual organs in male and female B6C3F1 mice after dietary exposure to turmeric oleoresin for 103 weeks (NTP, 1993)

Sex	Site	Tumour morphology	mg/kg of feed	Incidence[a]	Historical controls
M	Liver	Hepatocellular adenoma	0	25/50	11/50;7/50;21/50;17/50
			2000	28/50	
			10 000	35/50**	
			50 000	30/50	

Sex	Site	Tumour morphology	mg/kg of feed	Incidence[a]	Historical controls
M	Liver	Hepatocellular carcinoma	0	12/50	8/50;4/50;15/50;10/50
			2000	18/50	
			10 000	16/50	
			50 000	18/50	
M	Small Intestine	Carcinoma	0	0/50	0/50;1/50;2/50/0/50
			2000	3/50	
			10 000	3/50	
			50 000	0/50	
F	Liver	Hepatocellular adenoma	0	7/50	5/50;3/50;1/50;4/50;4/50
			2000	8/50	
			10 000	19/51**	
			50 000	14/50	
F	Liver	Hepatocellular carcinoma	0	7/50	0/50;2/50;3/50;1/50;1/50
			2000	5/50	
			10 000	10/50	
			50 000	6/50	
F	Pituitary Gland (Pars Distalis)	Adenoma	0	0/46	3/50;5/50;10/50;8/50
			2000	2/49	
			10 000	4/50	
			50 000	5/50*	

[a] Incidence was adjusted for mortality
*= $p<0.05$ or ** = $p < 0.01$ for paired comparisons between the control and the dosed groups.

Rats

F344/N rats (60 animals/sex/dose) were fed *ad libitum* diets containing 0, 2000, 10 000 or 50 000 mg/kg turmeric oleoresin (major component 79-85% curcumin compound I, CAS No 458-37-7) for 103 weeks, equal to daily doses of 0, 80/90, 460/440 or 2000/2400 turmeric oleoresin/kg bw/day, in males and females respectively.

The rats were housed 5 animals/cage and observed twice daily 7 days/week. Individual animal weights were recorded weekly for the first 13 weeks, then once every four weeks thereafter; food consumption was monitored by cage once every four weeks. An interim sacrifice of 10 randomly selected rats/group was conducted at 65 weeks, which included complete gross and microscopic evaluations, assay of a standard set of haematology and clinical chemistry parameters and weights of seven selected organs. At termination, a complete necropsy was performed on all animals, including both gross and microscopic evaluations.

No differences in survival rates between treated and control rats were observed. Survival rates ranged from 30-36% for males and 54-68% for females. No explanation for the lower survival rate in males compared to females was given; survival rates in treated males were similar to the survival rate in male controls. Hyperactivity was observed at the highest dose of curcumin during some observation periods. The final mean group body weights of the high-dose males and females were slightly less than the control's despite similar food intake. At 15 months, relative liver weights were significantly elevated in females fed 10 000 and 50 000 mg/kg. At 15 months for the 50 000 mg/kg groups, haematocrit, haemoglobin and red cells were significantly lower while platelet and reticulocytes (males only) were significantly higher.

In the gastrointestinal tract of high-dose male rats, the following benign neoplastic effects were reported: ulcers, hyperplasia and hyperkeratosis of the forestomach; ulcers, hyperplasia and inflammation of the caecum and colon; and sinus ectasia of the mesenteric lymph node. These lesions were considered likely to be regenerative and not neoplastic in nature. Benign neoplastic

gastrointestinal effects reported in high-dose female rats included ulcers, hyperplasia and inflammation of the caecum and sinus ectasia of the mesenteric lymph node. Neoplasms were not reported in males. In females, however, clitoral gland adenoma and carcinoma were reported (Table 5); however, the incidence of hyperplasia of the clitoral gland was similar in all groups of female rats. The marginal increase of clitoral gland adenoma was neither dose-related nor associated with a corresponding increase in hyperplasia (NTP, 1993 as cited by JECFA).

The conclusion of the NTP report was that there was no evidence of carcinogenic activity of turmeric oleoresin in male F344/N rats administered 2000, 10 000 or 50 000 mg/kg, but equivocal evidence of carcinogenic activity of turmeric oleoresin in female F344/N rats based on increased incidences of clitoral gland adenomas in the curcumin exposed groups (NTP, 1993).

Table 4: Incidence of primary tumours in individual organs (excluding mammary gland tumours) in female F344/N rats after dietary exposure to turmeric oleoresin for 104 weeks[b] (NTP, 1993). Historical control data were derived from NTP (NTP 2007).

Site	Tumour morphology	mg/kg of feed	Incidence[a]	Historical controls
Clitoral Gland	Adenoma	0	5/50	3/50;5/50;10/50;8/50
		2000	12/48	
		10 000	15/47*	
		50 000	16/49*	
Clitoral Gland	Carcinoma	0	1/50	1/50;3/50;0/50;2/50
		2000	4/48	
		10 000	0/47	
		50 000	0/49	

[a] Incidence was adjusted for mortality
* = $p<0.05$ for paired comparisons between the control and the dosed groups.
[b] No neoplasms were found in male rats.

No new chronic toxicity /carcinogenicity studies have been identified by the ANS Panel.

Although statistically significant increases in the incidences of hepatocellular adenomas (mid-dose males and females), small intestinal carcinomas (low- and mid-dose males) and pituitary gland adenomas (high-dose females) in mice and clitoral gland adenomas (females) in rats were observed (NTP, 1993), JECFA concluded that these effects were not dose-related and that curcumin was not a carcinogen. Gastrointestinal irritation (ulcers, hyperplasia and inflammation) was common in male and female rats in the high-dose group but this was not observed in mice. The NOAEL for gastrointestinal effects in rats was 10 000 mg/kg in the diet, equal to 440 mg/kg bw/day.

The Panel noted that all statistically significant effects noted by the NTP refer to benign neoplastic lesions (adenomas) and that the incidences for malignant neoplastic lesions (carcinomas), including the carcinomas of the small intestine in male mice, did not reach statistical significance. The Panel also noted that the effects observed were not dose-dependent, were in line with historical control values and were not consistent across sexes and/or species. The Panel noted moreover that hepatocellular tumours occurring in untreated and treated B6C3F1 mice are not relevant for humans. Therefore the Panel agrees with JECFA that curcumin is not carcinogenic.

3.2.5. Reproductive and developmental toxicity

JECFA evaluated two reproductive studies on turmeric and turmeric extracts (Garg, 1974; Bhavanishankar and Murthy, 1987), but these studies were not taken into account in their assessment

since the test material used in these studies is not comparable in composition to curcumin used as a colour additive in food.

A multigeneration study in Wistar rats was conducted according to OECD Testing guideline 416 (adopted on 26th May 1983) using curcumin comprising 80% curcumin (99% total curcuminoids). The study was conducted in India and the laboratory was inspected by European Inspectorates for compliance with EU and OECD guidelines, and the study was conducted in accordance with GLP. The study was reported to and evaluated by JECFA and subsequently published in the peer reviewed literature (Ganiger et al., 2007). Rats (30 animals/sex/dose) were fed diets containing curcumin at a concentration of 0, 1500, 3000 or 10 000 mg/kg of diet (equal to 0, 130–140, 250–290 or 850–960 mg/kg bw/day in males and 0, 160, 310–320 or 1000–1100 mg/kg bw/day in females) starting from 10 weeks before the mating period and throughout mating. Treatment of females continued throughout pregnancy and weaning of the offsprings. The total periods of treatment were 21 weeks for the parental generation and 24 weeks for the F1 generation. On postnatal day 4, the litter sizes of the F1 offspring were standardised to a maximum of eight animals. After weaning, 30 males and 30 females of the F1 generation were selected to become the parents of the F2 generation. Parents were observed for clinical signs, body weights, food intake, cohabitation interval and duration of gestation. Pups were weighed on postnatal days 1, 4, 7, 14 and 21. All parents, F1 weanlings not selected for mating and all F2 weanlings were subjected to complete necropsy at terminal sacrifice. The following indices were calculated: male and female fertility index, percentage of matings resulting in pregnancy, number of implantations, percentage of pregnancies resulting in birth of live litters, percentage of live pups born, post-implantation loss, mean litter size and mean viable litter size, live birth index and percentage survival of pups at postnatal days 4, 7, 14 and 21. Ovaries, uterus, vagina/cervix, testes, epididymides, seminal vesicles, prostate, coagulating glands, liver, kidney, pituitary and adrenals glands were examined histologically.

The study authors reported that there were no treatment-related clinical signs of toxicity, ophthalmological changes or mortality during the study and that during the premating period, there were no treatment-related effects in group mean body weights and net body weight gains or food consumption between treated and control animals of either generation. There were no differences in gestational or postpartum body weights or food consumption in either generation (Ganiger et al., 2007). During days 10–15 of gestation there was a dose-related decrease in body weight gain in the dams of the parental generation, which was statistically significantly different from that of controls (body weight gains, >80% than that of controls) at the intermediate and highest doses (JECFA, 2004; Ganiger et al., 2002). At this time, body weights were reported to be below the range of values for the historical controls. However, maternal body weights did not differ significantly between groups at the end of gestation. The mean body weights of the F2 offspring (both sexes combined) were significantly decreased on postnatal days 1 and 7 at the intermediate dose, and on postnatal days 7, 14 and 21 at the highest dose. A dose-related trend was apparent, but the effect was small, with average body weights being >90% that of the control pups, and the observed changes were reported to be within the range of the data for historical controls. There were no other effects on general health, body weight, pup survival and fertility indices in either generation. The effects at the intermediate dose were observed at isolated time-points only and were considered to be incidental. JECFA considered that the small body weight reduction in the F2 pups of the highest dose group prevented this dose level from being the NOAEL, and therefore the intermediate dose, equal to 250–320 mg/kg bw/day for the F1 generation, was considered by JECFA to represent the NOAEL. JECFA allocated an ADI for curcumin of 0-3 mg/kg bw/day based on the intake of 250-320 mg/kg bw/day in the mid-dose group as the NOAEL.

The study authors concluded that the NOAEL for reproductive toxicity of curcumin, fed in the diet for two successive generations to rats in this study was 10 000 mg/kg, which is equivalent to 847 and 959 mg/kg bw/day for male rats and 1043 and 1076 mg/kg bw/day for females for F0 and F1 generations, respectively.

The Panel considered the decreased body weight gain in the F2 generation observed at the highest dose levels an adverse effect and agrees with the NOAEL allocated by JECFA of 250-320 mg/kg bw/day.

3.2.6. Hypersensitivity and intolerance

Hypersensitivity

In the JECFA and SCF evaluations no studies related to intolerance/allergenicity were evaluated. In the TemaNord evaluation it is indicated that no relevant data on curcumin-induced food allergy or intolerance are present in the literature.

A recent case study is described in which a woman suffered from contact dermatitis after using a face-cream in which tetrahydracurcumin was employed. Tetrahydrocurcumin was identified as the ingredient responsible for the contact dermatitis. In this paper, reference is made to six more cases in which patients suffered from contact dermatitis caused by amongst others *C. longa*, curcumin in food colouring and/or tetrahydracurcumin (Thompson and Tan, 2006).

Two more cases of contact urticaria from curcumin are reported by Liddle et al. (2006).

The Panel noted that although several cases on contact dermatitis and urticaria are described in the literature, no relevant data on curcumin-induced food allergy or intolerance are present in literature. The case studies on contact dermatitis or urticaria are considered to be of little relevance for the evaluation of curcumin as a food additive.

The Panel also noted that curcumin was reported to have an inhibitory effect on histamine release from mast cells, and that results summarized for a murine model of allergy indicate a marked inhibition of allergic response in animals treated with curcumin, suggesting a major role for curcumin in reducing the allergic response (Kurup and Barrios, 2008).

Immunomodulation

Antony et al. (1999) investigated the immunomodulatory activity of curcumin in Balb/c mice. Balb/c mice (6 animals/group) were treated with five doses of liposome-encapsulated curcumin intraperitoneally. Curcumin administration was found to increase the total WBC count (15 290) significantly on the 12^{th} day Group of animals treated with vehicle alone showed results similar to that of normal animal (10 130 on 12^{th} day). Curcumin increased the circulating antibody titre (512) against SRBC. Curcumin administration increased the plaque-forming cells (PFC) in the spleen and the maximum number of PFC was observed on the 6^{th} day (1130 PFC/10(6) spleen cells) after immunization with SRBC. Bone marrow cellularity was enhanced by curcumin administration, from 12.9 x 10^6 cells/femur in the control, to 16.9 x 10^6 cells/femur in the curcumin treated group, although this effect was not statistically significant. Alpha-esterase positive cells were statistically significantly ($p<0.01$) increased from 1205/4000 cells in the control to 1622/4000 cells in the curcumin-treated group. A significant increase to 169% of the control in macrophage phagocytic activity was also observed in curcumin-treated animals ($P<0.001$). The authors concluded that these results indicate the immunostimulatory activity of curcumin.

South et al. (1997) reported the effects of dietary curcumin on three major types of immune function examined in rats. Immunoglobulin (IgG) production, delayed-type hypersensitivity and natural killer cell activity were evaluated after 5 weeks of dietary exposure to 1, 20 or 40 mg/kg bw/day of curcumin. The highest dose of curcumin significantly ($p<0.05$) enhanced anti-KLH IgG antibody production upon stimulation by injection of aqueous KLH, raising the response from an antibody titre

of 9.2 +/- 2.2 to 19.5 +/- 0.8. Rats receiving lower dietary concentrations (1 or 20 mg/kg) of curcumin were not statistically different in IgG production from rats receiving no curcumin in their diet. Neither delayed-type hypersensitivity nor natural killer cell activity was different from control values at any dietary concentration of curcumin.

The immunological effect of oral administration of food colouring agents was recently studied in rats given oral doses of 157.5 mg curcumin/kg bw for 4 weeks (Hashem et al., 2010). After 2 weeks all animals were immunostimulated by intraperitoneal injection of sheep red blood cells 10% (1 ml/rat). Results revealed that the treatment had no effect on the body weight gain. The authors concluded that curcumin exerted a suppressing effect on the cellular but not on the humoral immune response. However, the effects reported, such as a decrease in circulating neutrophiles and monocytes, an increase in lymphocytes and a decreased delayed hypersensitivity, were limited and when statistically significant, only at $p<0.05$. Total serum protein, albumin, total globulin and albumin/globulin ratio were not affected. The Panel considered that the biological significance of these data is limited.

3.2.7. Other studies

Human studies

JECFA evaluated two studies in humans. In a clinical trial (also described in section 3.1), 15 patients with advanced colorectal cancer receiving Curcuma extract daily for up to 4 months were physically examined and blood samples were taken on days 1, 2, 8 and 29 of treatment and monthly thereafter. Blood samples were analysed for total blood cell count and concentrations of urea, electrolytes and markers of liver and bone function. Curcuma extract was administered at a dose equivalent to 26, 72, 108, 144 or 180 mg of curcumin/day, with three patients receiving each dose. The only adverse effects reported were gastrointestinal symptoms. During the first month of treatment one patient receiving curcumin at a dose of 108 mg/day experienced nausea, which resolved spontaneously without discontinuing the treatment. Two patients, who received curcumin at a dose of 72 or 180 mg/day, respectively, experienced diarrhoea. In the absence of controls, and in view of the clinical conditions of the patients, it is not clear whether these symptoms were related to treatment (Sharma et al., 2001).

Twenty-five patients with conditions indicating a high risk of malignancy were given curcumin (purity, 99.3%) for 3 months. The starting dose was 500 mg/day, which was increased stepwise to 1000, 2000, 4000, 8000 and finally 12 000 mg/day. The patients received regular follow-up, including physical examination, weekly haemogram, and measurement of blood electrolytes and biochemistry parameters every 2 weeks. No adverse effects were reported at doses up to 8000 mg/day. The highest dose of 12 000 mg/day was not acceptable to the patients because of the bulky volume of the tablets (Cheng et al., 2001).

Two more recent papers, one describing a study with a curcumin formulation and the other describing a study with curcumin were available to the Panel for evaluation. A dose escalation study (also described in section 3.1) for a curcuminoid formulation (containing a minimum of 95% of three curcuminoids: curcumin, bisdemethoxycurcumin, and demethoxycurcumin; dose levels from 500-12000 mg) was performed with 24 healthy human volunteers. Seven of the 24 healthy human volunteers experienced only minimal toxicity (diarrhoea, headache, rash and/or yellow stool) that did not appear to be dose-related, whereas the other volunteers did not experience adverse effects (Lao et al., 2006).

In a review of Hsu and Cheng (2007), it is stated that in phase I clinical studies, curcumin at doses up to 3600-8000 mg daily for 4 months did not result in discernible toxicities except mild nausea and diarrhoea.

Based on the three studies with human volunteers described above, for dose levels up to 12 000 mg/day, only short-term and semi-chronic adverse effects, such as gastrointestinal effects, headache and rash were observed, but without clear dose-relationships.

In a study described by Joshi et al. (2003) nine healthy volunteers between 20 and 33 years of age were tested for haemoglobin, blood counts, liver and kidney functions, bleeding and clotting time and serum electrolytes initially and at 1 and 3 months of treatment. They were administered 0.6 ml of turmeric oil (TO) three times a day for 1 month and 1 ml in 3 divided doses for 2 months. The acute tolerability study on Day 1 was conducted in a Clinical Pharmacology Day Care Unit. Blood pressure and pulse were recorded frequently on Day 1 and at 24, 48, 72 and 96 hours and fortnightly till 12 weeks. Volunteers were daily supervised for TO intake as well as for any side effects throughout the study period. Of the nine volunteers enrolled for the study, one discontinued on the 3rd day for allergic skin rashes which, on discontinuation of TO, gradually disappeared after two weeks. Another discontinued on the 7th day for intercurrent fever requiring antibiotic treatment. Seven volunteers completed the study. There was no effect of TO, in two doses, on pulse and blood pressure and no side-effects in acute tolerability study on Day 1. There was no effect of TO intake on weight, blood pressure, symptoms and signs up to 12 weeks. There was no clinical, haematological, renal or hepatic toxicity of TO at 1 month and 3 months. Serum lipids did not show significant change except in one volunteer (reversible).

Other studies

Dance-Barnes et al. (2009), studied lung tumour promotion by curcumin in a transgenic mouse model for lung cancer that expresses the human Ki-ras (G12C) allele in a doxycycline (DOX) inducible and lung specific manner. The effects of curcumin were compared with the lung tumour promoter, butylated hydroxytoluene (BHT), and the lung cancer chemopreventive agent, sulindac. DOX was given in the drinking water (500 g/ml) beginning 8 weeks after birth. BHT was administered 1 week after initiation of DOX treatment and consisted of six weekly intraperitoneal injections of 150 mg/kg of BHT in olive oil. Control mice received olive oil vehicle at a dose of 0.5 ml/25 g. Separate groups of mice were fed either the chemopreventive agent sulindac at a dose of 80 mg/kg diet or 4000 mg/kg diet (the latter being equivalent to 600 mg/kg bw/day) of curcumin starting 2 days after the initiation of DOX. Treatment of DOX-induced mice with dietary curcumin increased tumour multiplicity (36.3 +/- 0.9 versus 24.3 +/- 0.2) and progression to later stage lesions, results which were similar to animals that were co-treated with DOX/BHT. Microscopic examination showed that the percentage of lung lesions that were adenomas and adenocarcinomas increased to 66% in DOX/BHT, 66% in DOX/curcumin and 49% in DOX/BHT/curcumin-treated groups relative to DOX only treated mice (19%). Immunohistochemical analysis also showed increased evidence of inflammation in DOX/BHT, DOX/curcumin and DOX/BHT/curcumin mice relative to DOX only treated mice. In contrast, co-treatment of DOX/BHT mice with 80 mg/kg ppm of sulindac inhibited the progression of lung lesions and reduced the inflammation. Lung tissue from DOX/curcumin-treated mice demonstrated a significant increase (33%, P = 0.01) in oxidative damage, as assessed by the levels of carbonyl protein formation, relative to DOX-treated control mice after 1 week on the curcumin diet. The authors concluded that these results suggest that curcumin may exhibit organ-specific effects to enhance reactive oxygen species formation in the damaged lung epithelium of smokers and ex-smokers.

Also studies on antioxidant/prooxidant activity of curcumin in *in vitro* models with various cells have been reported (Banerjee et al., 2008; Kunwar et al., 2008; McNally et al., 2007) but these studies do not add to the re-evaluation of the safety of curcumin as an additive.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Curcumin (E 100) is a dicinnamoylmethane food dye consisting of three principal colouring components. The product consists essentially of curcumins i.e. the colouring principle (1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione and its desmethoxy- and bis-desmethoxy-derivatives.

Curcumin has been evaluated by the Scientific Committee for Food (SCF) in 1975. No ADI was set by SCF as they considered that curcumin (from natural foods) could be classified as colour for which an ADI could not be established but which is nevertheless acceptable for use in food. The SCF (1975) identified several gaps in the database at the time of their evaluation and stated that metabolic studies in several species and if possible in man, adequate long-term studies in another species, reproduction and embryotoxicity including teratogenicity studies would be needed if considerable extension of use in food of this colour was contemplated in the future.

In 2004, JECFA allocated an ADI of 0-3 mg/kg bw/day, based on the NOAEL of 250-320 mg/kg bw/day in the multigeneration study in rats and the application of an uncertainty factor of 100 (JECFA 2004).

Specifications for curcumin have been defined in Directive 2008/128/EC and by JECFA (JECFA, 2006). The purity is specified as not less than 90% total colouring matters. Specifications define 90% of the material of commerce. From the definition it may be assumed that the remaining 10% may be accounted for by minor amounts of oils and resins naturally occurring in turmeric, but this is never explicitly stated. The Panel noted that the ratio between the three principal colouring components in curcumin is variable and not specified.

The Panel also noted that specifications for curcumin according to Directive 2008/128/EC and JECFA (2006) differ with regard to the solvents that are allowed for extraction and purification of curcumin and their maximum levels in the material of commerce and with regard to the maximum allowed lead concentration (which is given as ≤ 10 mg/kg and ≤ 2 mg/kg, respectively) and with regard to other metals. Furthermore, no details were found as to the precise chemical nature of the curcumin aluminium lake.

Regarding the kinetics of curcumin, in animal studies it appeared that curcumin is rapidly metabolised and excreted (mainly via faeces). Data are somewhat contradictory with respect to the absorption of curcumin. Some studies reported little absorption, while a study with tritium-labelled material indicated an absorption of somewhere approximately 60% of an oral dose administered. It is not clear from the study summary if the tritium label was stably included in the molecule. Nevertheless, hepatic clearance, including biliary excretion, is sufficiently efficient to prevent a major fraction of the curcumin dosed from entering the systemic circulation. After intravenous administration in animals, major biliary metabolites were the glucuronides of tetrahydrocurcumin and hexahydrocurcumin, with dihydroferulic acid and ferulic acid being present as minor metabolites. Based on discolouration of fur, it may be concluded that at high dose levels curcumin may accumulate in the body, but it is noted that fur discolouration may also have been caused by contact of the fur with the curcumin in the food. In humans, blood serum and plasma levels of curcumin are very low after administration of doses up to 2000 mg of curcumin and curcumin is excreted mainly via the faeces. Curcumin was detected in the serum after administration of doses > 4000 mg of curcumin (approximately 60 mg/kg bw).

Studies in humans also reveal that it is unlikely that substantial concentrations of curcumin occur in the body after ingestion at doses up to 12 000 mg/person, equivalent to 200 mg/kg bw for a 60 kg person.

The acute toxicity of curcumin is low, but this information is considered of little relevance for its safety evaluation for use as a food colorant. The short-term toxicity of curcumin also appears to be low. In the 6-day rat study of Gupta et al. (1980), already available at the time JECFA allocated the ADI, gastric erosion following curcumin administration in the diet was observed, but this was not observed in 13-week studies (NTP, 1993), in which the NOAEL with respect to gross and microscopic pathological changes was 9280 and 7700 mg/kg bw/day in female and male mice respectively, and 2760 and 2587 mg/kg bw/day in female and male rats respectively, which were the highest doses tested. The NTP described that hyperplasia of the mucosal epithelium was observed in the caecum and colon of male and female rats that received the highest dose level of curcumin, but these effects were apparently not considered to be adverse (NTP, 1993). The Panel derived a NOAEL for the 90-day study in rats of 2.5% curcumin in the diet, equivalent to 1300 mg/kg bw/day for males and 1450 mg/kg bw/day for females, based on hyperplasia at the highest dose level.

Curcumin was studied for genotoxicity in a battery of short-term assays of genetic toxicity. The compound was not active in the following assays: *Salmonella*/microsome test using strains TA98 and TA100, SCE using hamster lung fibroblasts and human embryo fibroblasts. Neither curcumin nor commercial turmeric oleoresin (containing 17.5% of curcumin) were active in the *Salmonella*/mammalian microsome test using TA1535, TA100 and TA98 strains (Jensen, 1982). Curcumin was reported not to induce chromosome damage in CHO cells *in vitro* (Au and Hsu, 1979). The results of several other genotoxicity studies on turmeric or curcumin revealed no mutagenic activity in bacteria treated with turmeric preparations containing up to 85% curcumin (NTP, 1993).

Positive results were reported in the rec assay (*B. subtilis*) and for chromosomal aberrations in hamster lung fibroblasts (Kawachi et al., 1980). A 79-85% purity preparation induced chromosomal aberrations and SCEs *in vitro* (NTP, 1993). Blasiak et al. (1999a, 1999b) provided evidence that curcumin induced DNA damage (measured as DNA-strand breaks in the Comet assay) in human lymphocytes and gastric mucosa cells *in vitro* when present in the low micromolar range (10-50 µM) and furthermore demonstrated that curcumin works in an additive fashion with hexavalent chromium, a well known mutagen and carcinogen. Antunes et al. (1999) substantiated this finding in that curcumin was found to induce DNA damage in CHO cells at a concentration of 10 µM and potentiated the effect of doxorubicin, a known free radical generator.

In an *in vivo* study in mice injected *i.p.* with curcumin of unknown purity there was some evidence of SCE induction at low frequency at concentrations above 25 mg/kg bw, while in rats fed curcumin of unknown purity there was equivocal evidence for the induction of chromosomal aberrations (Giri et al., 1990). The Panel noted that the curcumin tested in these *in vivo* tests was of unknown purity.

Weanling Swiss albino mice fed control diets or diets containing 0.5% turmeric (curcumin content unknown) or 0.015% curcumin of unknown purity (equivalent to 20 mg/kg bw/day) for 12 weeks were used in several genetic toxicity tests (Vijayalaxmi, 1980). Groups of eight females given curcumin or turmeric exhibited no effect in the micronucleus test. Groups of five males and five females given turmeric or curcumin showed no cytogenetic effect on the bone marrow chromosomes. Similarly no effect of the substances was noted in a dominant lethal study in which 15 male and 45 female mice were exposed to the test diets (Vijayalaxmi, 1980).

In 1996, JECFA concluded that no genotoxicity studies with high purity curcumin were available and that in limited studies with curcumin preparations of up to 85% purity, or of unknown purity, no mutagenic activity was seen in bacteria and only equivocal activity was seen in assays for the induction of chromosomal aberrations. JECFA concluded that there was no evidence to show that curcumin was genotoxic. In the last evaluation by JECFA, (2004) no new studies were evaluated.

The TemaNord evaluation (2002) stated that no *in vitro* genotoxicity studies are available with high-purity curcumin. Employing curcumin preparations of a purity of up to 85%, no mutagenicity has been observed in the Ames assay or in assays studying chromosomal aberrations. Furthermore, curcumin potentiated the effect of doxorubicin, a known free radical generator, which is in agreement with the finding by Blasiak et al. (1999a; 1999b).

Several new *in vitro* genotoxicity studies are available since these previous evaluations. Curcumin induced DNA damage (measured in the Comet assay)(Cao, 2006), damage in both mitochondrial and nuclear DNA in HepG2 cells (Cao et al., 2006) and a small but significant increase in micronuclei in HepG2 cells (Cao et al., 2007).

Also a new *in vivo* study in rats is available. Curcumin spice caused a statistically significant dose-dependent increase in the number of MNPCEs and in the frequencies of total chromosomal aberrations over the control in male rats which received a suspension of curcumin spice (not further specified) corresponding to 0.5, 5, 10, 25 and 50 mg/kg bw in 1 ml distilled water orally, daily, for four weeks (El-Makawy and Sharaf, 2006).

In Long-Evans Cinnamon (LEC) rats, exposure to 0.5% curcumin (95% purity) in the diet enhanced etheno-DNA adduct formation 9- to 25-fold in nuclear DNA and 3- to 4-fold in mitochondrial DNA. LEC rats are a model for human Wilson's disease and develop chronic hepatitis and liver tumours owing to accumulation of copper and induced oxidative stress (Nair et al., 2005).

The Panel considered that the indications provided by the positive results for curcumin in several *in vitro* and *in vivo* tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded, and that the available *in vivo* genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity.

In 1993, the NTP reported the results of long-term carcinogenicity studies in which rats and mice were exposed to levels of 0, 2000, 10 000 or 50 000 mg/kg diet turmeric oleoresin (79%-85% curcumin) for 103 weeks. For the rat study, NTP concluded that there was no evidence of carcinogenic activity of turmeric oleoresin in male F344/N rats, but equivocal evidence of carcinogenic activity of turmeric oleoresin in female F344/N rats based on increased incidences of clitoral gland adenomas in the exposed groups. For mice, the NTP (1993) concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin in male B6C3F1 mice based on a marginally increased incidence of hepatocellular adenoma at the 10 000 mg/kg diet level, and the occurrence of carcinomas of the small intestine in the 2000 and 10 000 mg/kg diet groups (although the Panel noted that the incidences were not statistically significant). For female B6C3F1 mice, the NTP concluded that there was equivocal evidence of carcinogenic activity of turmeric oleoresin based on an increased incidence of hepatocellular adenomas in the 10 000 mg/kg diet group. JECFA concluded that these effects were not dose-related and that curcumin is not a carcinogen.

The Panel noted that all statistically significant effects noted by the NTP refer to benign neoplastic lesions (adenomas) and that the incidences for malignant neoplastic lesions (carcinomas), including the small intestine carcinomas of male mice, did not reach statistical significance. The Panel also noted that the effects observed were not dose-dependent, were in line with historical control values and were not consistent across sexes and/or species. The Panel noted moreover that hepatocellular tumours occurring in untreated and treated B6C3F1 mice are not relevant for humans. The Panel also noted that the absence of dose-related effects in the NTP study is not due to saturating absorption kinetics because the data demonstrated that blood plasma concentrations increased linearly in a dose-related manner over the dietary concentration range of 0.1 to 2.5%, and that plasma levels of curcumin tended to plateau only at the higher dietary level of 5.0%. The Panel agrees with JECFA that curcumin is not carcinogenic. The Panel also concluded that this eliminates the concerns over genotoxicity.

In the NTP (1993) studies, gastrointestinal irritation (ulcers, hyperplasia and inflammation) was common in male and female rats in the high-dose group but this was not observed in mice. The NOAEL for gastrointestinal effects in rats was 10000 mg/kg in the diet, equal to 440 mg/kg bw/day.

Regarding the reproductive toxicity of curcumin, JECFA concluded on the basis of a multigeneration study in rats that were fed with curcumin for periods of up to 24 weeks that the NOAEL was 250–320 mg/kg bw/day since decreased body weight gain in the F2 generation was observed at doses equal to 960–1100 mg curcumin/kg bw/day. However, this study was published in 2007 by Ganiger et al. and the authors defined the highest dose as the NOAEL in contrast to the conclusion of the JECFA that

was published before. The current JECFA ADI of 0-3 mg/kg bw/day is based on this NOAEL of 250-320 mg/kg bw/day with application of an uncertainty factor of 100.

The Panel considered the decreased body weight gain in the F2 generation observed at the highest dose level as an adverse effect and agrees with the NOAEL allocated by JECFA of 250-320 mg/kg bw/day.

Based on this NOAEL, which is lower than the NOAEL of 440 mg/kg bw derived from the 2-year NTP study in rats, and an uncertainty factor of 100, the Panel derives an ADI of 3 mg/kg bw/day.

The exposure assessment approach goes from the conservative estimates that form the First Tier of screening, to progressively more realistic estimates that form the Second and Third Tier. The dietary exposure to curcumin from the MPL's of use was estimated by the Panel using the Budget method (Tier 1) with the assumptions described in the report of the SCOOP Task 4.2. The Panel calculated a theoretical maximum daily exposure of 6.9 mg/kg bw/day for adults, and 11.9 mg/kg bw/day for a typical 3 year-old child.

Refined exposure estimates have been performed both for children and the adult population according to the Tier 2 and Tier 3 approaches described in the SCOOP Task 4.2, which combines, respectively, detailed individual food consumption information from the population with the MPL's of use as specified in the Directive 94/36/EC on food colours (Tier 2), and with the maximum reported use levels of curcumin, as identified by the Panel from the data by Industry (Tier 3).

For children (1-10 years old), estimates have been calculated from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Sweden, Czech Republic, Greece, Cyprus and Germany). For the adult population, the Panel has selected the UK population as representative of the EU consumers for curcumin exposure estimates.

When considering MPL's (Tier 2), estimates reported for the UK adult population give a mean dietary exposure of 0.9 mg/kg bw/day and 3.3 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'non-alcoholic flavoured beverages'). The mean dietary exposure of European children (aged 1-10 years) ranged from 0.5 to 3.8 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile.

When considering the maximum reported use levels (Tier 3), estimates reported for the UK adult population give a mean dietary exposure to curcumin of 0.8 mg/kg bw/day and 2.0 mg/kg bw/day for high level consumers (mean consumption plus intake at the 97.5th percentile of 'non alcoholic flavoured beverages'). The mean dietary exposure of European children (aged 1-10 years) ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.1 to 7.1 mg/kg bw/day at the 95th percentile.

The Panel noted that at the maximum levels of use of curcumin, intake estimates for 1- to 10-year old children at the mean and the high percentiles (95th) are above the ADI of 3 mg/kg bw/day in some European countries.

This exposure assessment is however not taking into account the use of turmeric (*Curcuma longa)* as a spice in cooking, and this may contribute substantially to the dietary exposure of curcumin. The use of turmeric as a spice added to foods and used in home-made recipes was assessed using data from Irish adults (aged 18-64 years) and children (aged 5-12 years). Intakes of curcumin from turmeric as a spice consumed by adults (consumers only, n=66) ranged from a mean intake of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile, while intakes of curcumin based on the intake of curry powder (consumers only, n=91) also ranged from a mean intake of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile. In children, intakes of curcumin from turmeric as a spice (consumers only, n=7) ranged from a mean intake of 0.1 mg/kg bw/day to 0.2 mg/kg bw/day at the 97.5th percentile, while intakes of curcumin based on the intake of curry powder (consumers only, n=21) ranged from a mean intake of curcumin of 0.1 mg/kg bw/day to 0.3 mg/kg bw/day at the 97.5th percentile. Therefore, it can

be concluded that the combined exposure to curcumin through its use as a food colour and as a spice will exceed the ADI for both adults and children, especially at the higher percentiles.

The Panel noted that the aluminium lake of the colour may lead to an anticipated aluminium exposure of up to 3.84 mg/kg bw/week (in UK pre-school children, for high level consumers of panned and compressed confectionery), which exceeds the Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week (EFSA, 2008), and that therefore specifications for the maximum level of aluminium in the lakes may be required.

CONCLUSIONS

Curcumin (E 100) is a dicinnamoylmethane dye authorised as a food additive in the EU and was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974, 1978, 1980, 1982, 1987, 1990, 1992, 1995, 2000, 2002 and 2004 and the EU Scientific Committee on Food (SCF) in 1975. The SCF concluded in 1975 that an Acceptable Daily Intake (ADI) could not be established but that curcumin was nevertheless acceptable for use in food. In 2004 JECFA allocated an ADI of 0-3 mg/kg bw/day.

Curcumin consists of three principal colouring components. It consists essentially of curcumins i.e. the colouring principle (1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione and its desmethoxy- and bis-desmethoxy-derivatives.

The Panel concluded that the present database supports an ADI of 3 mg/kg bw/day based on the NOAEL of 250-320 mg/kg bw/day from the reproductive toxicity study for a decreased body weight gain in the F2 generation observed at the highest dose level, and an uncertainty factor of 100.

The Panel concluded that at the maximum levels of use of curcumin, intake estimates for 1- to 10-year old children at the mean and the high percentiles (95^{th}) are above the ADI of 3 mg/kg bw/day in some European countries.

When the exposure assessment takes into account the use of turmeric (*Curcuma longa)* as a spice in cooking in one country (Irish adults and children), it was found to contribute to the dietary exposure of curcumin in consumers of the spice. Therefore, it can be concluded that the combined exposure to curcumin through its use as a food colour and as a spice will exceed the ADI for both adults and children, especially at the higher percentiles.

The Panel concluded that intake from the normal diet amounts to less than 7% of the ADI of 3 mg/kg bw/day, resulting from an average exposure to curcumin of 0.1 mg/kg bw/day from the intake of turmeric and curry powder each for both children and adults.

The purity of curcumin is specified as not less than 90% total colouring matters. The Panel noted that the specifications for curcumin should be updated to define the residual 10%.

The Panel noted that specifications for curcumin according to Commission Directive 2008/128/EC and JECFA differ with regard to the solvents that are allowed for extraction and purification of curcumin and their maximum levels in the material of commerce and with regard to the maximum allowed lead concentration (which is given as ≤ 10 mg/kg and ≤ 2 mg/kg respectively) and with regard to other metals.

The Panel noted that the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
2. CIAA (Confederation of the Food and Drink Industries of the EU), 2009. CIAA data in response to the Commission request for data: "EFSA re-evaluation of food colours" - Southampton study colours), (SANCO/E3/OS/km D 53007, May 22, 2009).
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ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

1. **Decision rules taken to deal with QS authorisations for MPL: (see the decision tree in Figure 1)**

    a. If the category 'All other foodstuff' is QS, the highest observed MPL value should be used, which is 500 mg/kg

    b. At the food category level, if a colour is authorised QS in a food category for one or more colours

        i. If a value is available for only one colour, this value is used for all the colours

        ii. If many values are available for more than one colour, the highest value is used

        iii. If there is no available value, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

Particular cases:

- **Edible casings QS**: If available use the pork-based products use level; if there is no value available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds**: The MPL of 100 mg/kg (from the flavoured processed cheese category) is used, except for E 120 (Cochineal) whose level is 125 mg/kg for red marbled cheese.

2. **Rules to identify the maximum reported use levels to be used for the refined exposure assessment:**

    A maximum reported use level is the maximum value selected from reported usage by industry and analytical data provided to the Panel:

    a. If the identified maximum reported use level is greater than or equal to the actual MPL, then the actual MPL is used by default.

    b. If both maximum analytical and maximum current use level data are available, priority is given to the use level data, even if analytical values are lower or higher; the selected value is rounded to the nearest whole number.

    c. If no use level data are available, because either no uses were reported or industry was not asked to provide them, the choice is made between the highest analytical value or the MPL:

        i. if more than10 analytical data are available, the highest quantified reported value is used;

ii. if less than10 analytical data are available, the MPL is used.

d. If the highest use level or the highest analytical data are higher than the proposed adjusted QS values for MPL, priority is given to the highest use level/analytical data.

3. Tiered approach to intake estimation

The basic principles of the stepwise approach for the estimation of additives' intakes involve, for each successive Tier, a further refinement of intakes from the conservative estimates for screening (Tier 1) to more realistic estimates (Tier 2 and 3) (EC, 2001). Depending on the information on use levels data available, the three screening tiers approach must be adapted (see Figure 2 for the decision rules).

The three screening tiers performed both for children and adult population are:

Tier 1: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours and the Budget method.

Tier 2: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours with adjustment for quantum satis usages, and national individual food consumption data.

Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.

In Tier 2 and 3, the following approach is used to calculate the high percentile consumption: The high consumption should be calculated by examining the 97.5th percentile of food additive intake per food group, and selecting the highest intake* and then adding this value to the sum of the mean intakes for the remaining food groups. This approach is slightly different to the usual approach, in which the two highest food group intakes at the 97.5th percentile of additive intakes are added to the mean consumption of the other food groups. The approach was modified based on evaluation of the EXPOCHI study, as it provides a more realistic estimate of exposure.

*High consumption value of Fruit wines (still or sparkling), Cider (except cidre bouche) and perry, Aromatized fruit wines, cider and perry from UK adult data is not taken into account for the calculation of high percentile exposure when this food category appeared to be the highest P95 exposure. In this case the second highest contributor is taken in the calculation.

GLOSSARY/ABBREVIATIONS

ACh	Acetylcholine
ADI	Acceptable Daily Intake
ADHD	Attention-Deficit/Hyperactivity Disorder
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
ANS	Panel on Food Additives and Nutrient Sources added to Food
BHT	Butylated Hydroxytoluene
CAS	Chemical Abstracts Service
ChEs	Cholinesterases
CHO	Chinese hamster ovary cells
CI	Confidence Interval
CIAA	Confederation of the Food and Drink Industries of the EU
DOX	Doxycycline
EC	European Commission
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FAO/WHO	Food and Agriculture Organization/World Health Organization
FSA	UK Food Standard Agency
FSAI	Food Safety Authority of Ireland
GHA	Global Hyperactivity Aggregate
GLP	Good Laboratory Practice
HMPC	Herbal Medicinal Products
HPLC	High-Performance Liquid Chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KLH	Keyhole Limpet Haemocyanin
LEC	Long-Evans Cinnamon
LOAEL	Lowest-Observed-Adverse-Effect Level
LOD	Limit of Detection
LOQ	Level of Quantification
MNPCE	Micronucleated Polychromatic Erythrocyte
MPL	Maximum Permitted Level

NATCOL	Natural Food Colours Association
NOAEL	No-Observed-Adverse-Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PND	Post-Natal Day
PTWI	Provisional Tolerable Weekly Intake
SCE	Sister Chromatid Exchanges
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
SCF	Scientific Committee for Food
TO	Turmeric Oil
TWI	Tolerable Weekly Intake
UNESDA	Union of European Beverage Associations