COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of hepatotoxicity of dietary turmeric supplements

1. In the context of the recent hepatis outbreaks related to the consumption of dietary turmeric supplements, this paper reviews the hepatotoxicity of turmeric and provides a UK exposure assessment in relation to the ADI for curcumin (the active ingredient).

Brief history of recent hepatitis outbreaks

2. Between December 2018 and 20th July 2019, a total of 21 individual cases of acute cholestatic hepatitis "likely to be linked to the consumption of food supplements based on curcumin and piperine" were reported on Italian territory [1]. A total of 18 turmeric supplements have been associated with this hepatitis outbreak, one of which ("Curcuma Liposomal & black pepper" by Nutrimea) was recalled by Belgium's Federal Agency for Food Chain Safety (AFSCA) [2].

3. Whilst the AFSCA said that "the exact source of contamination had not yet been established" [2], an update from Italy's National Institute of Health does not appear to mention contamination of the curcumin supply as a cause for the hepatitis [1]. The Institute said "the interdisciplinary group, section dietetics, and the technical committee for animal nutrition and health concluded that, to date, the causes are likely to be related to individual susceptibility, pre-existing alterations, latent hepato-biliary function or even the use of drugs". The Institute adopted a warning for the labelling of the supplements in question (to take effect from 31st December 2019), advising against their use for subjects with altered hepato-biliary function, and recommending medical advice when other medications are being taken. The Institute adds that for turmeric powder, which was implicated in one hepatitis case, no particular recommendations are needed especially considering its history of consumption as a food.

4. Contamination of turmeric supplements has been of health concern in the past. For example, the FSA recently received an enquiry where a family had been consuming raw turmeric (grown on lead-rich Indian soil) for over a year; following a blood test, the enquirer tested positive for lead. Furthermore, in March 2009, a brand of turmeric-based supplements called Fortodol (also sold as Leppin Miradin) was associated with 11 liver-related adverse event reports in Sweden and a further five in Norway, including deaths. The supplement was found to contain nimesulide, a

[1] https://www.nutraingredients.com/Article/2019/07/26/Italy-rejects-contamination-as-hepatitis-cause-citing-individual-susceptibility

[2] https://www.nutraingredients.com/Article/2019/07/11/Belgium-recall-same-curcumin-based-supplement-linked-to-Italian-hepatitis-cases

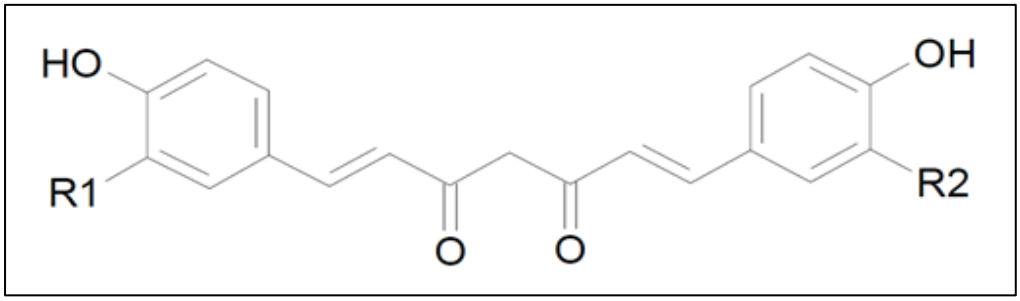
nonsteroidal anti-inflammatory drug known to cause liver problems [3]. Turmeric powder can be adulterated with powders of other species of Curcuma (Reyma et al. 2004). The powder of Curcuma zedoaria, a common adulterant in turmeric powder, is known to be toxic; the high-protein flour of C. zedoaria caused 100 % mortality within 6 days when given at 320 g/kg diet to 5 week-old rats (Latif et al. 1979). The identity of questionable samples of turmeric powders can be examined under light and/ or scanning electron microscopy in comparison with authentic samples (Li et al. 2011), in addition to DNA based techniques (Remya et al. 2004).

Background of turmeric supplements

5. Turmeric is the common name for the rhizome (underground stem) of Curcuma longa L., a perennial herb cultivated in tropical and subtropical regions of the world. India is the largest producer of turmeric, supplying over 90 % of the world's demand (Olojede et al. 2009). There are about 70 varieties of C. longa cultivated in India (Sasikumar 2005). For centuries, turmeric has been widely used for imparting colour and flavour to food, and in Indian and Chinese traditional medicine as a remedy for the treatment of inflammation and other diseases (Ammon & Wahl 1991). Its antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer properties have been reviewed by Hewlings & Kalman 2017.

6. Many of the pharmacological properties of turmeric have been attributed to curcumin, hereafter referred to as diferuloylmethane. These properties include antioxidant, analgesic, anti-inflammatory, antiseptic, anticarcinogenic, chemopreventive, chemotherapeutic, antiviral, antibacterial, antifungal and antiplatelet activities (Alok et al. 2015). Diferuloylmethane is a polyphenol compound naturally present within turmeric rhizomes. Its derivatives demethoxycurcumin and bisdemethoxycurcumin are also present within turmeric rhizomes. These compounds are collectively called "curcuminoids" and their chemical structures are presented in Figure 1.

Figure 1: Chemical structures of the principal curcuminoids present in turmeric



The principle colouring component is diferuloylmethane (also known as curcumin, R1 = R2 = OCH3; MW = 368.4 g/mol). Derivatives of diferuloylmethane are also present which include demethoxycurcumin (R1 = OCH3, R2 = H; MW = 338.4 g/mol) and bisdemethoxycurcumin (R1 = R2 = H; MW = 308.3 g/mol).

[3] https://www.nutraingredients.com/Article/2009/03/23/Tainted-turmeric-supplements-linked-to-Scandinavian-deaths

7. The relative proportions and total concentration of curcuminoids within turmeric rhizomes vary depending on the variety grown and the conditions of cultivation (Li et al. 2011) (Table 1).

Table 1: Percentage composition of curcuminoids in turmeric powders and oleoresin extracts (adapted from Li et al. 2011).

Curcuminoid	**Composition in turmeric powders (mean) (% dry weight)**	**Composition in turmeric oleoresin extracts (mean ± s.d.) (% dry weight)**
Diferuloylmethane	2.86	19.51 ± 2.07
Demethoxycurcumin	1.47	8.31 ± 1.13
Bisdemethoxycurcumin	1.36	6.22 ± 0.88
Total	5.69	34.04 ± 4.08

8. Curcuminoids can be extracted from ground turmeric powder using organic solvents to create a turmeric oleoresin extract (Table 2). JECFA (1992) lists several solvents permitted for extraction: acetone, methanol, ethanol, and isopropanol. The European Commission, however, has a different list of permitted solvents: acetone, carbon dioxide, ethyl acetate, dichloromethane, n-butanol, methanol, ethanol, and hexane (EC 2008). According to JECFA specifications, residual solvent concentrations in turmeric oleoresin intended for use in food are limited to 25 mg/kg for hexane, 30 mg/kg for acetone, dichloromethane, and 1,2-dichloroethane, and 50 mg/kg for ethanol, methanol, and isopropanol (JECFA 1992). The extraction methodology used affects the curcuminoid content (37-55 %) (Li et al. 2011), and the essential oil content (< 25%) (Braga et al. 2003) of the turmeric oleoresin (Table 2).

Table 2: Preparation and composition of turmeric products that are commercially available as dietary supplements (adapted from Li et al. 2011).

Commercial product name	**Preparation**	**Composition**
Turmeric powder	Prepared from dried rhizomes of C. longa	0.58-3.14 % diferuloylmethane (dry weight), and other curcuminoids
Turmeric oleoresin extract	Treat turmeric powder with organic solvents	37-55 % curcuminoids, < 25 % essential oil
Turmeric oil extract	Treat turmeric powder with steam distillation or supercritical CO_2 extraction	Essential oil from leaves usually dominated by monoterpenes whilst oil from rhizomes mainly contains sesquiterpenes
Curcumin powder	Purify turmeric oleoresin through crystallisation	> 90 % curcuminoids, and minor amounts of essential oil

9. Whilst curcuminoids are responsible for the yellow-orange colour of turmeric, it is the volatile sesquiterpenes present in the rhizome's essential oil that are responsible for its aroma and taste (Li et al. 2011). The major sesquiterpenes in turmeric oil extract are α-, β-, and Ar-turmerone (Li et al. 2011), which can together account for > 40 % of the essential oil present in turmeric rhizomes (Stanojević et al. 2015). Turmerone possesses diverse pharmacological activities that include antioxidant and antimutagenic activities (Jayaprakasha et al. 2002). Turmeric oil extract can be prepared in various ways, for example through the treatment of turmeric powder with steam distillation, supercritical CO_2 extraction (Li et al. 2011), or by evaporating the organic solvent of a crude turmeric oleoresin extract (Funk et al. 2010) (Table 2).

10. Diferuloylmethane powder can be obtained through the purification of turmeric oleoresin by crystallisation (Li et al. 2011). However, there can be limited commercial availability of authentic samples of pure diferuloylmethane, since its separation from demethoxycurcumin and bisdemethoxycurcumin can be difficult and time consuming. Thus, commercial "pure" diferuloylmethane is, in many cases, a mixture of at least these three curcuminoids (Li et al. 2011). For example, a sample of commercial "pure" diferuloylmethane (labelled as 94 % purity) was, after HPLC analysis, found to be of about 70 % purity (Li et al. 2011). In addition, the composition of a sample of commercial "curcumin" was found to be approximately 71.5 % diferuloylmethane, 19.4 % demethoxycurcumin, and 9.1 % bisdemethoxycurcumin (Pfeiffer et al. 2003).

11. "Curcumin" powder is authorised for use as a colouring agent in food (E 100), where its purity is specified as "not less than 90 % total colouring matters" (i.e. diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin) (EC 2008). Moreover, JECFA (1992) stated that "purified extracts of turmeric containing more than 90 % total colouring matter are subject to specifications for 'Curcumin'"(JECFA 1992). EFSA (2010) noted that the specification should be updated to define the composition of the remaining 10 %, which may be accounted for by minor amounts of turmeric essential oil. Directive 94/36/EC states the maximum permitted levels (MPLs) for E100 in foodstuffs (which range from 20 to 500 mg/kg depending on the food item) and beverages (which range from 100-200 mg/L) (EC 1994).

12. The FSA's Novel Foods Team consider turmeric food supplements comprising of turmeric oleoresin extract or pure curcumin powder to be novel. These products were not significantly used as a food or food ingredient before 15th of May 1997. Therefore, before these products may be placed on the market in the EU as a food supplement, a safety assessment under the Novel Food Regulation is required. Unauthorised foods may be removed from the market for example if adverse health effects are indicated.

Health-based guidance values

13. In 1975, the SCF evaluated curcumin. 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.

14. In 1995, JECFA evaluated the results of toxicology and carcinogenicity studies in rats and mice administered turmeric oleoresin containing 79-85 % curcuminoids (NTP 1993). After 15 months of treatment, absolute and relative liver weights were increased in both male and female mice in the mid- and highest-dose groups relative to controls. The NOEL for liver enlargement was 2000 mg/kg in the diet, equal to 220 mg/kg bw/ day. On the basis of the NOEL of 220 mg/kg bw/day in the carcinogenicity study of mice and a safety factor of 200, JECFA increased the temporary ADI to 0-1 mg/ kg bw and extended it, pending the submission of the results of a reproductive toxicity study with curcumin (JECFA 1995).

15. In 2004, JECFA noted that the turmeric oleoresin used in the NTP (1993) study did not comply with the current specification for curcumin. JECFA withdrew the temporary ADI and established an ADI for curcumin of 0-3 mg/kg bw based on significant decreases in the average bodyweights of Wistar rat F2 generation pups (paragraph 37) (JECFA 2004).

16. In 2010, EFSA concluded that the present database supports an ADI of 3 mg/kg bw/day, also based on significant decreases in the average bodyweights of Wistar rat F2 generation pups (EFSA 2010).

ADME

17. Following an oral administration of 1 g/kg bw “curcumin” to rats, up to about 75 % of the curcumin was excreted in the faeces, whilst negligible amounts of curcumin appeared in the urine (Wahlstrom & Blennow 1978). The study authors concluded that measurements of blood plasma levels and biliary excretion showed that curcumin itself was poorly absorbed from the gut. Oral bioavailability is similarly low in humans (paragraphs 38-40), due to poor absorption and extensive first-pass metabolism in the intestine and liver (Ireson et al. 2001, 2002). For example, in 12 cancer patients receiving 450-3600 mg of diferuloylmethane daily for 1 week prior to surgery, low levels (near the detection limit of 3 nM) of diferuloylmethane and its glucuronide and sulphate conjugates were found in samples of peripheral or portal blood taken intraoperatively; these compounds were not found in the bile or liver tissue in any of the patients (Garcea et al. 2004).

18. The enzymatic hydrolysis of plasma samples taken from rats orally administered curcuminoids showed that the predominant metabolites in plasma were glucuronide and sulfate conjugates of diferuloylmethane (Asai & Miyazawa 2000). Ravindranath & Chandrasekhara (1981) evaluated the tissue distribution of “curcumin” using tritium-labelled drug in male Wistar rats. They found that radioactivity was detectable in blood, liver, and kidney following doses of 10, 80, or 400 mg of [^{3}H]curcumin. With 400 mg, considerable amounts of radiolabelled products were present in tissues 12 days after dosing. The percentage of curcumin absorbed (60-66 % of the given dose) remained constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption. Shoba et al. (1998) orally administered 2 g/kg bw “curcumin” to rats and reported the absorption and elimination half-lives to be 0.31 ± 0.07 and 1.7 ± 0.5 hours, respectively. In humans, however, the same dose of curcumin did not allow the

calculation of these half-life values because the serum curcumin levels were below the detection limit at most of the time points in most of the experimental subjects.

Exposure assessment

Human exposure through the diet

19. EFSA estimated dietary exposure to diferuloylmethane in children and adults using national consumption data with maximum permitted levels (MPLs) specified in Directive 94/36/EC (EC 1994) (tier 2 approach), and maximum reported use levels (tier 3 approach). Estimates of dietary exposure to diferuloylmethane obtained from these approaches are presented in Table 3.

20. For adults (> 18 years old), the EFSA Panel estimated the exposure based on the UK consumption survey as the UK population is considered to be one of the highest consumers of soft drinks in Europe and individual food consumption data (UK NDNS, 2000-2001) are available (Tennant 2006, 2007). For children (1-10 years old), the Panel estimated exposure based on dietary exposure assessments for children in Europe (EXPOCHI) project. The EXPOCHI project details individual food consumption data from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Sweden, Czech Republic, Cyprus, Greece and Germany). As the UK is not included in the EXPOCHI consortium, estimates for UK children (1.5 - 4.5 years old) were made by the Panel with the use of individual food consumption data (UK NDNS, 1992-1993) (Tennant 2006, 2007).

Table 3: Estimates of dietary exposure to curcumin in the UK adult population and in children from the EXPOCHI study and UK.

	UK adult exposure (> 18 years old) to curcumin (mg/kg bw/day)	**Children (UK & EXPOCHI , 1-10 years old) exposure to curcumin (mg/kg bw/day)**
Maximum permitted level (tier 2):		
Mean exposure	0.9	0.5-3.8
Exposure 97.5th %ile	3.3	1.2-7.2
Maximum reported use levels (tier 3):		
Mean exposure	0.8 (1.0 *)	0.5-3.4 (0.7-3.6 *)
Exposure 97.5th %ile	2.0 (2.6 *)	1.1-7.1 (1.6-7.6 *)

* Includes dietary exposure to diferuloylmethane from turmeric powder added to food as a spice and curry powder (see Table 4).

21. In tier 2, the main contributor to curcumin exposure from the UK adult diet was non-alcoholic flavoured drinks (46 %). The main contributors to the estimates of mean curcumin exposure for UK children (and children considered by the EXPOCHI consortium) were non-alcoholic beverages (13-55 %), fine bakery wares (e.g. biscuits, cakes, wafer) (12-43 %), desserts, including flavoured milk products (12-45 %), and sauces, seasonings, pickles, relishes, chutney and piccalilli (11-42 %).

22. In tier 3, the main contributor to curcumin exposure from the UK adult diet was non-alcoholic flavoured drinks (50 %). The main contributors to the estimates of mean curcumin exposure for UK children (and children considered by the EXPOCHI consortium) were fine bakery wares (e.g. biscuits, cakes, wafer) (13-47 %), desserts (including flavoured milk products) (13-52 %), non-alcoholic beverages (15-57 %) and sauces and seasonings (11-45 %).

23. The exposure assessment in tier 3 does not take into account the use of turmeric as a spice in cooking. The addition of turmeric spice in cooking was estimated to contribute to the dietary exposure of diferuloylmethane by the EFSA panel (Table 4).

Table 4: Estimates of dietary exposure to diferuloylmethane from ingestion of spice added to food and curry powder in adults in children.

	Adult (18-64 years old) diferuloylmethane exposure (mg/kg bw/day)	**Children (5-12 years old) diferuloylmethane exposure (mg/kg bw/day)**
Exposure from spice added to food *		
Mean	0.1 (n = 66)	0.1 (n = 7)
97.5th %ile	0.3 (n = 66)	0.2 (n = 7)
Exposure from curry powder added to food *		
Mean	0.1 (n = 91)	0.1 (n = 21)
97.5th %ile	0.3 (n = 91)	0.3 (n = 21)

* 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). The dietary intake of curry powder was also considered, as turmeric powder is a widespread ingredient in it (approximately 30 % depending on the blend).

Human exposure through turmeric supplements

24. In addition to exposure to diferuloylmethane through a normal diet, turmeric supplements can also be taken. Ground turmeric powder, turmeric oleoresin extract and diferuloylmethane powder are the compositions of turmeric that contain diferuloylmethane and which can be taken as supplements. A selection of these supplements (which can be readily purchased on the internet) are presented in Table 5, where the contribution of daily supplemental intake of diferuloylmethane to the ADI of 3 mg/kg bw/ day is calculated, with and without dietary diferuloylmethane. Of the supplements presented in Table 5, those containing ground turmeric powder do not exceed the ADI of 3 mg/kg bw/day for diferuloylmethane, either alone or with dietary intake of diferuloylmethane. Conversely, supplements comprising of turmeric oleoresin extract generally do lead to an exceedance of the ADI.

25. Different approaches have been taken to increase the in vivo bioavailability of diferuloylmethane when taken as a dietary supplement. These approaches include

modified formulations (e.g. NovaSOL® and Longvida®) and the use of additives (e.g. BioPerine®):

- NovaSOL® curcumin consists of micellar curcumin, which has been shown to increase bioavailability of curcumin in humans by 185 % (Schiborr et al. 2014).
- Longvida® Optimised Curcumin consists of solid lipid curcumin particles (SLCP). In healthy human subjects, the mean peak concentration of diferuloylmethane achieved from dosing 650 mg of SLCP was 22 ng/mL, whereas plasma curcumin from dosing an equal quantity of unformulated 95 % curcuminoid extract was not detected (limit of detection 1 ng/mL) (Gota et al. 2010).
- BioPerine® consists of piperine (an alkaloid derived from black pepper which is a known inhibitor of hepatic and intestinal glucuronidation). Concomitant oral administration of piperine has been shown to enhance the bioavailability of diferuloylmethane in humans by 2000 % (Shoba et al. 1998).

Table 5: The contribution of some commercial turmeric supplements to the dietary ADI for diferuloylmethane.

Commercial product name	**Main ingredient(s)**	**Daily dosage recommended on vendor website**	**Diferuloyl methane intake (mg/ kg bw day) (supplement only)** *	**% ADI of 3 mg/kg bw/day (supplement only)**	**% ADI of 3 mg/kg bw/day (supplement & diet) ‡**
Prowise Organic Turmeric Max Strength (Prowise Healthcare)	Turmeric powder, black pepper	1 capsule, each containing 600 mg turmeric powder	0.25	8.2 †	41.7
Nature's Garden Turmeric 400mg (Holland and Barrett)	Turmeric powder	2 capsules, each containing 400 mg turmeric powder	0.33	10.9	44.3
Turmeric Curcumin Supplement with Bioperine® (Primal Living)	Turmeric powder, Bioperine®	1-2 tablets, each containing 500 mg turmeric powder	0.41	13.6 †	47.0
Maxsorb Turmeric (Simply Supplements)	Turmeric powder, piperine	2 capsules, each containing 500 mg turmeric powder	0.41	13.6 †	47.0
Organic Turmeric (Curcumin) + Black Pepper Strong 600mg Capsules (Health Essentials)	Turmeric powder, BioPerine®	2 capsules, each containing 600 mg turmeric powder	0.49	16.3 †	49.7
Opti-Turmeric capsules (HealthSpan)	NovaSOL® curcumin (containing	1-2 capsules, each containing	0.49	16.4 †	49.7

	turmeric extract)	30 mg curcuminoids			
Organic Turmeric Curcumin 1380 mg with Organic Black Pepper & Organic Ginger (Vita Bright)	Turmeric powder, black pepper	2 capsules, each containing 630 mg turmeric powder	0.51	17.2 †	50.3
Swanson Turmeric (Healthy Monthly)	Turmeric powder	2 capsules, each containing 720 mg turmeric powder	0.59	19.6	53.0
Advanced Turmeric - Turmeric/Curcumin with Bioperine® (Black Pepper) (Autoimmune Institute)	Turmeric powder, BioPerine®	2-4 capsules, each containing 500 mg turmeric powder	0.82	27.2 †	60.7
Cell Active Curcumin Plus (Cytoplan)	Longvida® Optimised Curcumin	2 capsules, each containing 250 mg Longvida® Optimised Curcumin	1.8	59.5 †	93.3
Flexi6 Gold (HealthSpan)	Turmeric extract	2 tablets, each containing 126 mg curcuminoids	2.1	68.8	103.3
Turmeric Tablets 20,000 mg (Nature's Best)	Turmeric extract	1 tablet containing 475 mg curcuminoids	3.9	129.6	163.3
Turmeric 500mg 95% Curcumin (20:1 extract eq. 10,000mg) + Bioperine® (Zip Fit)	Turmeric extract, BioPerine®	1 tablet containing 475 mg curcuminoids	3.9	129.6 †	163.3
High Potency Turmeric (Ancient Wisdom Modern Medicine)	Turmeric extract, Bioperine®	1-2 tablets, each containing 375mg curcuminoids	6.1	204.6 †	236.7
Turmeric 500 mg (HealthSpan)	Turmeric extract	1-2 tablets, each containing 475 mg curcuminoids	7.8	259.2	293.3
Turmeric 10,000 with BioPerine® Black Pepper Extract (HealthSpan)	Turmeric extract, BioPerine®	1-2 tablets, each containing 475 mg curcuminoids	7.8	259.2 †	293.3
Turmeric Pro with BioPerine® 12,500mg	Turmeric extract, BioPerine®	2 capsules, each containing	7.8	259.2 †	293.3

95% Curcuminoids (Evolution Slimming)		475 mg curcuminoids			
Curcumin (Turmeric) with Black Pepper - Powder Capsules (Supplement Place)	Turmeric extract, black pepper extract	2 capsules, each containing 750 mg curcuminoids	12.3	409.3 †	443.3
Turmeric + Bioperine® Tablets - 10,000mg)Just Vitamins)	Turmeric extract, BioPerine®	1-2 tablets, each containing 475 mg diferuloylmethane	13.6	452.4 †	486.7
Turmeric Curcumin Advanced Complex (Piping Rock Health Products)	Turmeric extract, black pepper extract	2-4 capsules, each serving of 2 capsules containing 1425 mg curcuminoids	23.5	783.1 †	816.7

* Calculated using adult body weight of 70 kg (EFSA 2012), and maximum recommended daily dosage. For turmeric powder, the calculation assumes 2.86 % is diferuloylmethane, e.g. (600 mg/day turmeric powder x 2.86 %) / 70 kg bw = 0.25 mg/kg bw/day. For turmeric oleoresin extract, calculation assumes 57.3 % of total curcuminoid content is diferuloylmethane (derived from Table 1). For Longvida® Optimised Curcumin, calculation assumes 25 % of the formulation is diferuloylmethane (Gota et al. 2010).
† Product formulation or composition expected to enhance bioavailability of diferuloylmethane.
‡ Assumes a mean contribution of 1.0 mg/kg bw/day diferuloylmethane from the diet for adults (see Table 3).

Review of hepatotoxicity of diferuloylmethane

Acute oral toxicity

26. In mice, oral LD_{50} values of 2 g/kg bw (unspecified % purity diferuloylmethane in extract) (Srimal & Dhawan 1973) and > 10 g/kg bw (turmeric extract estimated to contain about 79 % diferuloylmethane) were reported (Lilja et al. 1983).

27. In rats, oral LD_{50} values of 5 g/kg bw (“curcumin” suspended in arachis oil, purity not reported) (Wahlstrom & Blennow 1978) and > 10 g/kg bw (turmeric extract estimated to contain about 79 % diferuloylmethane) were reported (Lilja et al. 1983).

28. Liju et al. (2013) investigated the acute and subchronic toxicity of turmeric essential oil (TEO) from Curcuma longa L. Acute administration of TEO was done as a single oral dose of up to 5 g/kg bw, and a 13-week subchronic toxicity study was done at doses of 0.1, 0.25 and 0.5 g/kg bw/day in Wistar rats. There were no mortalities, adverse clinical signs or changes in body weight, water and food consumption during the acute and subchronic studies. Indicators of hepatic function such as aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were unchanged in treated animals compared to

untreated animals. Oral administration of TEO for 13 weeks did not alter total cholesterol, triglycerides, markers of renal function, serum electrolyte parameters and histopathology of tissues.

Subacute toxicity

29. Dietary turmeric powder (0.2 %, 1.0 %, 5.0 %, equivalent to 400, 2000, 10000 mg/kg bw/day) or ethanolic turmeric extract (ETE, 0.05 %, 0.25 %, equivalent to 100, 500 mg/kg bw/day) for 14 days, at doses reported to be cancer preventive in model systems, were found to be hepatotoxic in mice. The diferuloylmethane contents of the turmeric powder and ETE were not reported. Exposure of mice to dietary turmeric or ETE did not have any significant effect on body weight/ liver weight or liver to body weight ratios. Animals exposed to 0.2 % turmeric showed coagulative necrosis in liver. Livers from mice receiving 1.0 % and 5.0 % turmeric showed extreme degenerative changes with necrosis. Coagulative necrotic foci surrounded by a zone of regenerations were also evident. Similar changes were also seen in animals treated with 0.05 % and 0.25 % ETE. Necrotic changes in liver, the principal effect, was seen in 6/6 animals from 5.0 % turmeric, 3/6 from 1.0 % turmeric and 3/6 animals from 0.2 % turmeric. Similarly 3/6 animals from 0.05 % as well as 0.25 % ETE also showed these alterations. Liver from mice receiving control diet showed normal ultrastructure. In liver of the animals receiving 5.0 % or 1.0 % turmeric diet or 0.25 % or 0.05 % ETE, some of the parenchymal cells had round nuclei consisting of clumped or densely marginated chromatin. The cytoplasm consisted of numerous pleomorphic vacuolated mitochondria filled with dense bodies, surrounded by rough or smooth endoplasmic reticulum and free ribosomes, and an increased number of glycogen particles and Golgi complexes with vesicles. (Kandarkar et al. 1998).

Subchronic toxicity

Mice

30. Subchronic oral toxicity of turmeric and ethanolic turmeric extract (ETE) was studied in female Swiss mice and Wistar rats fed turmeric powder (0, 1 and 5 %) and ethanolic turmeric extract (0, 0.05 and 0.25 %) through the diet for 14 and/or 90 days. The curcuminoid content of the ETE was approximately 98%. The administration of a high dose of turmeric (5 %) for longer duration (90 days) showed a significant reduction in body weight gain, alterations in absolute and/ or relative liver weights, and hepatotoxicity i.e. focal necrosis or focal necrosis with regeneration both in mice and rats. In mice, lower doses of turmeric (i.e. 0.2 or 1 % for 14 days) also showed hepatotoxicity and they were found to be more vulnerable to turmeric-induced hepatotoxicity than rats. (Deshpande et al. 1998).

31. In a 13 week study, groups of 10 male and 10 female B6C3F_1 mice were fed diets containing 0, 1000, 5000, 10000, 25000, or 50000 ppm turmeric oleoresin, which were estimated to deliver average daily doses of 150, 750, 1700, 3850, or 7700 mg/kg bw to males and 200, 1000, 1800, 4700 or 9300 mg/kg bw to females (NTP 1993). The major component of the oleoresin was identified as diferuloylmethane (79 to 85 %). The percent composition was monitored periodically at the study laboratory with free-acid titration and high-performance liquid

chromatography methods, and no change in composition was observed. There were no deaths attributed to turmeric oleoresin and the final mean body weight gains and final mean body weights of all exposed groups of male and female mice were similar to those of the controls. Feed consumption by exposed male and female mice was similar to that by the controls. Absolute and relative liver weights of male mice that received 5000 ppm and male and female mice that received 10000, 25000 and 50000 ppm were significantly greater than those of the controls. Clinical findings in mice included stained fur, and discoloured faeces and urine. According to NTP, there were no biologically significant differences in hematologic, clinical chemistry, or urinalysis parameters, and there were no chemical related histopathologic lesions. EFSA (2010) concluded that the no-effect level with respect to gross and microscopic pathological changes was 7700 and 9280 mg/kg bw/day in males and females, respectively, which were the highest doses tested.

Rats

32. In a 28 day study, diferuloylmethane (purity > 98 %) was administered to Sprague Dawley rats through the diet at dose levels of 26.1, 84.8, 224,8, 459.7 and 1117.8 mg/kg bw/day. Clinical chemistry did not reveal major signs of liver damage associated with administration of diferuloylmethane. cDNA microarray experiments were performed on hepatic RNA. Diferuloylmethane altered the expression of 12 genes. Three 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 diferuloylmethane is a weak peroxisome proliferator in rats. (Stierum et al. 2008).

33. In a 13 week study, groups of 10 male and 10 female F344/N rats were fed diets containing 0, 1000, 5000, 10000, 25000, or 50000 ppm turmeric oleoresin (79 to 85 % diferuloylmethane), estimated to deliver average daily doses of 50, 250, 480, 1300, or 2600 mg/kg bw to males and 60, 300, 550, 1450, or 2800 mg/kg bw to females. All rats survived until the end of the study. The final mean body weight of males receiving 50000 ppm was 5 % lower than that of the controls. Feed consumption by exposed male and female rats was similar to that of controls. The absolute and relative liver weights of female rats and the relative liver weights of male rats receiving 5000, 10000, 25000, and 50000 ppm were significantly greater than those of the controls. According to NTP, these increases may have been due to mild hepatocellular swelling or hypertrophy. In the clinical chemistry, urinalysis, and other hematologic parameters, no differences were observed that were considered by the NTP to be biologically significant. Clinical findings included stained fur, and discoloured faeces and urine of exposed animals. Mild to moderate hyperplasia of the mucosal epithelium was observed in the cecum and colon of male and female rats that received 50000 ppm. (NTP 1993).

34. A six month toxicity study of curcuminoids extracted from the powdered dried rhizome of Curcuma longa was performed in six groups of 15 Wistar rats of each sex. The extract was reported to contain 58-67 % curcuminoids. The water control group received 5 ml of water/kg bw/day, while the 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 six months, but two weeks of no curcuminoids treatment elapsed before the time of sacrifice. It was found that 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 of 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. The EFSA Panel concluded that due to the lack of knowledge on the diferuloylmethane content and nature of the extract tested, the study could not be used to assess the safety of diferuloylmethane. (Chavalittumrong et al. 2002).

Chronic toxicity

2-year study in rats

35. Groups of 60 male and 60 female F344/N rats were fed diets containing 2000, 10000, or 50000 ppm turmeric oleoresin (79 to 85 % diferuloylmethane) for 104 (males) or 103 (females) weeks, which were estimated to deliver average daily doses of 80, 460, or 2000 mg/kg bw to males and 90, 440,or 2400 mg/kg bw to females. Nine or 10 rats from each exposure group were evaluated after 15 months. Survival of exposed male and female rats was similar to that of the controls (male: 0 ppm, 18/50; 2000 ppm, 17/50; 10000 ppm, 15/50; 50000 ppm, 17/50; female: 33/50, 27/50, 28/50, 34/50). The final mean body weights of all exposed male rats and female rats receiving 2000 and 10000 ppm were similar to those of the controls. The final mean body weights of male and female rats that received 50000 ppm were slightly lower (up to 10 %) than those of the controls throughout much of the study. Feed consumption by exposed male and female rats was similar to that by controls throughout the study. The absolute and relative liver weights of female rats receiving 10000 or 50000 ppm were significantly greater than those of controls at the 15-month interim evaluation. There were no clinical findings related to toxicity. In male and female rats receiving 50000 ppm the haematocrit values, haemoglobin concentrations and erythrocyte counts at the 15-month interim evaluation were significantly lower than those in the controls. In addition, platelet counts in male and female rats that received 50000 ppm and reticulocyte counts in male rats that received 50000 ppm were significantly higher than those in the controls. No biologically significant differences were observed in clinical chemistry parameters. (NTP 1993).

2-year study in mice

36. Groups of 60 male and 60 female B6C3F_1 mice were fed diets containing 2000, 10000, or 50000 ppm turmeric oleoresin (79 to 85 % diferuloylmethane) for 103 weeks, which were estimated to deliver average daily doses of 220, 520, or 6000 mg/kg bw to males and 320, 1620, or 8400 mg/kg bw to females (NTP 1993).

Nine or 10 mice from each exposure group were evaluated after 15 months. Survival of exposed male and female mice was similar to that of the controls (male: 0 ppm, 43/50; 2000 ppm, 43/50; 10000 ppm, 37/50; 50000 ppm 42/50; female: 39/50, 41/50, 34/50, 42/50). The mean body weight of female mice receiving 50000 ppm was slightly lower (up to 12 %) than that of the controls from about week 25. The final mean body weights of males that received 50000 ppm and females that received 10000 and 50000 ppm were significantly lower than those of controls. The final mean body weights of other exposed groups of male and female mice were similar to those of the controls. Feed consumption by exposed male and female mice was similar to that by the controls throughout the study. The absolute and relative liver weights of male and female mice receiving 10000 and 50000 ppm were significantly greater than those of the controls at the 15-month interim evaluation. There were no clinical findings related to toxicity. No biologically significant differences were observed in hematologic parameters. The alkaline phosphatase values of male and female mice that received 10000 and 50000 ppm were significantly higher than those of controls at the 15-month interim evaluation. The incidences of hepatocellular adenoma in male and female mice receiving 10000 ppm, but not those in mice receiving 2000 or 50000 ppm, were significantly increased (male: 25/50, 28/50, 35/50, 30/50; female: 7/50, 8/50, 19/51, 14/50). The number of male and female mice in the 10000 and 50000 ppm groups with multiple hepatocellular neoplasms compared with controls was of statistical significance. However, JECFA concluded that this effect was not dose-related and that curcumin is not a carcinogen (JECFA 1995). In addition, the EFSA Panel noted that all statistically significant effects noted by the NTP refer to benign neoplastic lesions (adenomas) (EFSA 2010). The EFSA Panel 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 $B6C3F_1$ mice are not relevant for humans. Therefore the Panel agreed with JECFA that curcumin is not carcinogenic.

Reproductive toxicity

37. In a multigeneration study of reproductive toxicity, Wistar rats were fed diets containing diferuloylmethane (> 90 % purity) at doses equal to 0, 130-140, 250-290 and 850-960 mg/kg bw/day in males, and 0, 160, 310-320 and 1000-1100 mg/kg bw/day in females (Ganiger et al. 2007). The total period of treatment was 21 weeks for the parental generation and 24 weeks for the F1 generation. No treatment-related clinical signs of toxicity during the study were reported. Significant decreases in the average weights of the F2 generation pups were observed at days 1 and 7 at the intermediate dose, and days 7, 14 and 21 at the high dose. These decreases represented < 10 % of the average weight of the concurrent controls and were reported to be within the range of the historical control data. There were no other effects on general health, body weight, pup survival or fertility indices in either generation. JECFA considered the effect on pup weight seen at the intermediate dose (equal to 250-320 mg/kg bw/day) to be incidental and therefore a NOEL. JECFA allocated an ADI for curcumin of 0-3 mg/kg bw/day based on the application of an uncertainty factor of 100 to this NOEL (JECFA 2004).

Human studies

38. Twenty-five patients with conditions indicating a high risk of malignancy were administered diferuloylmethane (purity 99.3 %) in tablet form for 3 months. The starting dose was 500 mg/day, which was increased stepwise to 1000, 2000, 4000, 8000 and finally 12000 mg/day (equivalent to 7, 14, 29, 57, 114, and 171 mg/kg bw/day). The patients received regular follow-up, including physical examination, weekly haemogram, and “biweekly” blood electrolytes and biochemistry study (though blood results not presented). No adverse effects were reported at doses up to 8000 mg/day. The highest dose of 12000 mg/day was not acceptable to the patients because of the bulky volume of the tablets. The serum concentration of curcumin usually peaked at 1 to 2 hours after oral intake of diferuloylmethane and gradually declined within 12 hours. The average peak serum concentrations after taking 4000 mg, 6000 mg and 8000 mg of curcumin were 0.51 ± 0.11 µM, 0.63 ± 0.06 µM, and 1.77 ± 1.87 µM, respectively. Urinary excretion of diferuloylmethane was undetectable. The study authors noted that these results suggest that diferuloylmethane is not adequately absorbed from the gastrointestinal tract. (Cheng et al. 2001).

39. Fifteen patients with advanced colorectal cancer received an extract of Curcuma (18 mg of diferuloylmethane and 2 mg of demethoxycurcumin suspended in 200 mg of essential oils derived from Curcuma spp.) daily for up to 4 months. The doses were equivalent to 36, 72, 108, 144 and 180 mg diferuloylmethane/day (equivalent to 0.5, 1.0, 1.5, 2.1, and 2.6 mg/kg bw/day), with three patients receiving each dose. Neither diferuloylmethane, or its metabolites (glucuronide or sulphate conjugates, or hexahydrocurcumin or hexahydrocurcuminol) were detected in plasma or urine. However, diferuloylmethane was detected in the faeces of all patients. Diferuloylmethane sulphate was also detected in the faeces of one of the patients receiving diferuloylmethane at a dose of 180 mg diferuloylmethane/day, which may have been a result of biotransformation in the gut. Thus, the study authors concluded that diferuloylmethane has low oral bioavailability in humans and may undergo intestinal metabolism. Oral Curcuma extract was well tolerated however, in the course of the study, one patient (receiving 108 mg diferuloylmethane/day) experienced nausea and two patients (receiving 72 and 180 mg diferuloylmethane /day) experienced diarrhoea. Dose-limiting toxicity was not observed (full blood cell count and urea, electrolytes, liver, and bone function were measured in venous samples). (Sharma et al. 2001).

40. In a subsequent phase I study, Sharma et al. (2004) evaluated another formulation, which was a 500 mg curcuminoid capsule containing 450 mg diferuloylmethane, 40 mg demethoxycurcumin, and 10 mg bisdemethoxycurcumin. The dose levels of diferuloylmethane were 450, 900, 1800, or 3600 mg/day for up to 4 months (equivalent to 6, 13, 26, and 51 mg/kg bw/day). A total of 15 patients with refractory colorectal cancer were enrolled. Full blood cell count and urea, electrolytes, liver, and bone function were measured in venous samples, and physical examination was performed, before treatment and on treatment days 1, 2, 8, 29, and monthly thereafter. The drug was well tolerated, however one patient consuming 450 mg diferuloylmethane daily and one patient consuming 3600 mg diferuloylmethane daily developed diarrhoea 1 month and 4 months into treatment, respectively. One patient consuming 900 mg diferuloylmethane daily experienced

nausea, which resolved spontaneously despite continuation of treatment. In addition, a rise in serum alkaline phosphatase level was observed in 4 patients, and serum lactate dehydrogenase rose to > 150 % of pre-treatment values in 3 patients. Diferuloylmethane was detected in plasma and urine samples from patients consuming 3600 mg of diferuloylmethane daily, but not from patients on the lower doses. Diferuloylmethane was detected in plasma samples taken 1 hour post-dose from 3 patients consuming 3600 mg of diferuloylmethane daily at a concentration of 11.1 ± 0.6 nmol/L. Analysis of urine suggested the presence of diferuloylmethane (0.1 - 1.3 µmol/L) and its sulfate (19 - 45 nmol/L) and glucuronide (210 - 510 nmol/L) conjugates in all of the samples from patients consuming 3600 mg of diferuloylmethane daily. Diferuloylmethane was not detected in plasma or urine from patients who received lower doses of diferuloylmethane. Abundant amounts of diferuloylmethane were recovered from the faeces at all of the dose levels. Diferuloylmethane levels in day 8 faecal samples from patients consuming 3600 mg of diferuloylmethane daily were between 25 and 116 nmol/g dried faeces.

41. A dose escalation study was performed with 24 healthy human volunteers using single doses of between 500-12000 mg of a turmeric extract (containing a minimum of 95 % of diferuloylmethane (75 %), bisdemethoxycurcumin (2 %), and demethoxycurcumin (23 %), equivalent to 7-171 mg/kg bw). Seven volunteers experienced minimal toxicity at doses > 14 mg/kg bw (diarrhoea, headache, rash and/or yellow stool) that did not appear to be dose-related. The remaining volunteers did not experience adverse effects (Lao et al. 2006).

42. 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 extract (TO; 59 % turmerone and Ar-turmerone) three times a day for 1 month and 1 ml in 3 divided doses for 2 months. One volunteer discontinued on day three for allergic skin rashes which, on discontinuation of TO, gradually disappeared by two weeks. Another discontinued on the day seven for intercurrent fever requiring antibiotic treatment. Seven volunteers completed the study. 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 hepatotoxicity of TO at 1 month and 3 months. Serum levels of AST, ALT, ALP, albumin, direct and indirect bilirubin were normal initially and remained within normal limits during treatment. One volunteer showed normal serum triglycerides and low-density lipoprotein (LDL) initially and at 4 weeks but elevated levels at 12 weeks. This volunteer was followed up for 1 month after discontinuation of TO and serum lipids returned to normal. (Joshi et al. 2003).

43. Kanai et al. (2011) evaluated the safety and feasibility of combination therapy using “curcumin” with gemcitabine-based chemotherapy. The ”curcumin” used was a mixture of diferuloylmethane (73 %), demethoxycurcumin (22 %), and bisdemethoxycurcumin (4 %), provided in microbead form. Gemcitabine-resistant patients with pancreatic cancer received 8 g oral curcumin daily (equivalent to 114 mg/kg bw) in combination with gemcitabine-based chemotherapy. The primary endpoint was safety for phase I and feasibility of oral curcumin for phase II study. Twenty-one patients were enrolled. No dose-limiting toxicities were observed in the phase I study and oral curcumin 8 g/day was selected as the recommended dose for

the phase II study. No patients were withdrawn from this study because of the intolerability of curcumin, which met the primary endpoint of the phase II study, and the median compliance rate of oral curcumin was 100 % (range 79-100 %). Plasma curcumin levels ranged from 29 to 412 ng/ml in five patients tested. Adverse effects were reported (neutropenia, fatigue, drowsiness, anorexia, obstruction of the gastrointestinal tract, and oedema) all of which were also attributed to disease progression or gemcitabine-based chemotherapy, and considered to be irrelevant to curcumin. Kanai et al. (2011) concluded that combination therapy using 8 g oral curcumin daily with gemcitabine-based chemotherapy was safe and feasible in patients with pancreatic cancer and warrants further investigation into its efficacy.

Clinical trials using turmeric preparations - EMA Summary

44. In September 2018, the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) considered several clinical studies with turmeric preparations involving patients with digestive disorders. Although a possible effect in improving symptoms was seen, firm conclusions could not be drawn due to limitations in the study design, for example only one study compared turmeric with a control group. However, the HMPC considered the effectiveness of these preparations for relief of mild digestive problems to be plausible and there is evidence that they have been used safely in this way for at least 30 years (including at least 15 years within the European Union). Thus, the HMPC concluded that turmeric preparations (which cover turmeric ground powders and extracts) can be used (only by adults) for relief of mild problems with digestion, such as feelings of fullness, slow digestion and flatulence. The EMA considered that side effects of dry mouth, flatulence and stomach irritation may occur. If symptoms continue for longer than two weeks or worsen while taking the medicine, a qualified healthcare practitioner should be consulted.

Human cases of hepatotoxicity

Case 1 (Melbourne, Australia; Luber et al. 2019)

45. A 52 year old woman presented to her general practitioner with a one-week history of nausea, pruritus, and painless jaundice with associated pale stools and dark urine. This occurred approximately one month following commencement of one tablet per day of Ancient Wisdom Modern Medicine High Potency Turmeric (375 mg curcuminoids and 4 mg black pepper per tablet), along with a flaxseed oil supplement and occasional diclofenac use for arthritic pain. (N.b. this supplement leads to an intake of 204.6 % of the ADI of 3 mg/kg bw/day, see Table 5). She had no prior history of liver disease and had normal liver function tests (LFTs) three months before. Her medical history was notable only for oligoarticular osteoarthritis.

46. Upon admission, all oral medications and supplements were ceased. She was found to have a bilirubin of 162 μmol/L with a hepatocellular profile on liver function tests (ALT 2591 U/L, AST 1770 U/L, ALP 263 U/L, and GGT 370 U/L). With progressive jaundice over the subsequent days she was referred to the emergency department, at which point her bilirubin peaked at 536 μmol/L.

47. Due to lack of significant improvement by day four of admission, a liver biopsy was performed. Histology showed nonspecific inflammatory changes with generally preserved hepatic architecture and no fibrosis. She was discharged day 12 of admission (bilirubin 260 μmol/L, ALT 1232 U/L) with the presumptive diagnosis of diclofenac induced liver injury. By two months after admission her liver function tests had normalised (bilirubin 21 μmol/L, ALT 33 U/L) and she was discharged from the clinic.

48. At this point she recommenced the turmeric supplement (1125 mg curcuminoids per day) as sole therapy for her arthritis. Three weeks later her nausea recurred and repeat liver function tests showed an acute hepatitis (ALT 2093 U/L, AST 1030 U/L, and bilirubin 60 μmol/L). She was advised to cease the turmeric supplement, and two months later her liver function tests had again normalised.

49. The turmeric supplements were sent for analysis by a validated liquid chromatography mass spectrometry method. Results were compared to a toxicology library containing approximately 1400 compounds, including medications, illicit drugs, and over-the-counter medicines. A further sample was analysed by inductively coupled plasma mass spectrometry for the presence of trace elements. The turmeric supplement tested negative for drugs, adulterants, or toxic heavy metals.

Case 2 (Melbourne, Australia; Luber et al. 2019)

50. A 55 year old man at routine check-up was found to have an asymptomatic transaminitis. His background history included idiopathic thrombocytopenic purpura, hypertension, gout, and osteoarthritis, with regular medications including long-term telmisartan, atenolol, and lercanidipine. He had no known liver history with normal liver function tests one year prior. His only new medication was commencement of a turmeric supplement five months prior to testing.

51. He was referred to a hepatologist and underwent a screen for causes of acute hepatitis. Abdominal ultrasonography showed diffuse steatosis. A drug reaction was suspected, and the turmeric supplement was ceased.

52. Follow-ups occurred over the subsequent four months. Near normalisation of liver function tests occurred by one month (ALT 96 U/L, bilirubin 10 μmol/L) with further improvement by four months after cessation (ALT 46 U/L, bilirubin 11 μmol/L). The turmeric supplement was the presumed cause of the hepatitis. The turmeric supplement was not known, thus further analysis on it could not be performed.

Case 3 (New York, USA; Suhail et al. 2019)

53. A 61 year old female with polycystic liver disease presented with fatigue, dark urine and polyarthralgias for one week. She denied alcohol use. Physical examination demonstrated right upper quadrant abdominal tenderness. Laboratory findings were notable for aspartate aminotransferase 1553 mg/dL, alanine aminotransferase 2607 mg/dL, alkaline phosphatase 246 mg/dL and total bilirubin 1.6 mg/dL with a direct component of 1 mg/dL. Hepatic synthetic function was intact. Medications included naproxen and ergocholecalciferol with no changes four years

prior to the onset of transaminitis. Viral infections were ruled out. Autoimmune work-up yielded positive antinuclear antibody (1:250) with normal anti-smooth muscle antibody and serum IgG levels. Abdominal Doppler was negative for portal or hepatic vein thrombosis. Magnetic resonance cholangiopancreatography did not show any biliary ductal pathology. It was discovered that the patient was taking turmeric supplements for 6 months. A liver biopsy demonstrated panlobular hepatitis with early parenchymal collapse suggestive of a morphologic counterpart of acute hepatitis and hepatocellular pattern of injury. The patient was thought to have drug-induced liver injury from turmeric pills that were discontinued and she was discharged with prednisone. Her LFTs normalized after 3 weeks, after which, her prednisone was tapered off.

54. Suhail et al. (2019) noted that the temporal association of liver injury in the patient, normalisation of LFTs upon withdrawal and improvement with steroids implicate the turmeric supplement as the likely causative agent of liver injury. The Roussel-Uclaf Causality Assessment Method (RUCAM) scale, which attempts to codify causality of drug toxicity into objective criteria, was eight in our patient indicating a probable adverse drug reaction. A negative serological workup and normalisation of LFTs following the discontinuation of steroids further solidify this conclusion.

Case 4 (Arizona, USA; Lukefahr et al. 2018)

55. A 71 year old woman at routine check-up was found to have an asymptomatic transaminitis. Her medical history included hypothyroidism, Raynaud's syndrome, osteoarthritis, hypertension, dyslipidaemia, irritable bowel syndrome, and diverticulosis, with regular medications including amlodipine, metoprolol, and atenolol. A 'low cost' turmeric supplement (of unknown product identification) was taken according to label recommendations for a period of 8 months prior to transaminitis testing.

56. She was referred to a gastroenterologist. Laboratory and biopsy findings led to a diagnosis of autoimmune hepatitis. Treatment was limited to withdrawing use of turmeric supplements. AST and ALT decreased significantly within 30 days of discontinuation, and normalised by 13 months. In this case, the patient (not the clinicians) hypothesised that the turmeric may have been the cause of the elevated liver transaminases and elected to cease its use.

57. Subsequent reassessment of the liver biopsy by the authors revealed autofluorescent inclusions in the pigment-laden histiocytes, with an excitation/emission spectrum consistent with curcumin, or possibly lipofuscin. Histiocyte fluorescence, which was not noted in liver biopsy specimens from patients with unrelated disorders, was quenchable by treatment with Sudan Black B (SBB), as has been reported for lipofuscin. However, because the authors also documented complete SBB quenching of curcumin autofluorescence in fixed cultured cells specifically loaded with curcuminoids, the authors could not ascertain with certainty whether the histiocyte inclusions here were composed of lipofuscin, a lysosomal degradation product and/or curcuminoids derived from the turmeric supplement that the patient was still consuming at the time of the biopsy.

Summary

58. The literature review of human studies suggests oral diferuloylmethane in humans is well tolerated up to doses of 114 mg/kg bw/day, though minor symptoms of nausea or diarrhoea may occur. Long-term studies are lacking, however. The UK exposure assessment for dietary turmeric supplements indicates substantial exceedances of the ADI can occur for diferuloylmethane, which are generally for the supplements containing turmeric extracts.

Questions on which the views of the Committee are sought

59. Members are invited to consider the following questions:

- Do Members have any comments on the animal toxicity data for turmeric or diferuloylmethane?
- Do Members have any comments on the recent case reports of human hepatotoxicity?
- In light of the above, does the Committee wish to reconsider the ADI that is currently based on reproductive toxicity?
- Given that the composition or formulation of turmeric supplements may substantially enhance the in vivo bioavailability of diferuloylmethane, does the Committee wish to advise on the extent of exceedance against the current ADI for supplemental diferuloylmethane that would be considered to represent a health risk?
- Given past reported contamination issues with turmeric supplements, would there be value in undertaking chemical analysis of turmeric supplements available on the UK market?
- Do Members have any other comments on this paper?

Secretariat

September 2019

References

Alok A., Singh I.D., Singh S., et al. (2015) Curcumin – Pharmacological Actions And its Role in Oral Submucous Fibrosis: A Review. Journal of Clinical and Diagnostic Research **9:** 1-3

Ammon H.P. & Wahl M.A. (1991). Pharmacology of Curcuma longa. Planta Medica **57:** 1-7

Asai A. & Miyazawa T. (2000) Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. Life Sci. **67:** 2785-93

Braga M.E.M., Leal P.F., Carvalho J.E., et al. (2003) Comparison of Yield, Composition, and Antioxidant Activity of Turmeric (Curcuma longa L.) Extracts Obtained Using Various Techniques. J. Agric. Food Chem. **51:** 6604-6611

Chavalittumrong P., Chivapat S., Rattanajarasroj S., et al. (2002). Chronic toxicity study of curcuminoids in rats. The Songklanakarin Journal of Science and Technology **24:** 633-647

Cheng A.L., Hsu C.H., Lin J.K., et al. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Research **21:** 2895-2900

Deshpande S.S., Lalitha V.S., Ingle A.D., et al. (1998) Subchronic oral toxicity of turmeric and ethanolic turmeric extract in female mice and rats. Toxicol Lett **95:** 183-193

EC (1994) European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 273, 10.9.94 Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31994L0036&from=EN

EC (2008) European Commission Directive 2008/128/EC Laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1 p. 20-63. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008L0128

EFSA (2010) EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the re-evaluation of curcumin (E 100) as a food additive. EFSA Journal **8:** 1679

EFSA (2012) Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal **10:** 2579

EMA (European Medicines Agency) (2018) Committee on Herbal Medicinal Products (HMPC) Assessment report on Curcuma longa L., rhizome. Available at: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-curcuma-longa-l-rhizoma-revision-1_en.pdf

Funk J.L., Frye J.B., Oyarzo J.N., et al. (2010) Anti-Arthritic Effects and Toxicity of the Essential Oils of Turmeric (Curcuma longa L.) J Agric Food Chem. **58:** 842-849

Garcea G., Jones D.J. L., Singh R., et al. (2004) Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. Br. J. Cancer **90:** 1011-1015

Ganiger S., Malleshappa H.N., Krishnappa H., et al. (2007) Two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats. Food and Chemical Toxicology **45:** 64-69

Gota V.S., Maru G.B., Soni T.G., et al. (2010). Safety and Pharmacokinetics of a Solid Lipid Curcumin Particle Formulation in Osteosarcoma Patients and Healthy Volunteers. J. Agric. Food Chem. **58:** 2095-2099

Harrington K.E., Robson P.J., Kiely M., et al. (2001). The North/South Ireland Food Consumption Survey: survey design and methodology. Public Health Nutrition **4:** 1037-1042

Hewlings S.J. & Kalman D.S. (2017) Curcumin: A Review of Its' Effects on Human Health Foods **6:** 92

Hsu C.H. & Cheng A.L. (2007). Clinical studies with curcumin. Advances in Experimental Medicine and Biology **595:** 471

Ireson C.R., Orr S., Jones D.J., et al. (2001) Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. Cancer Res **61:** 1058-64

Ireson C.R., Jones D.J., Orr S., et al. (2002) Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. Cancer Epidemiol Biomarkers Prev **11:** 105-11

IUNA (Irish Universities Nutrition Alliance) (2005). National Children's Food Survey. Main report: https://www.iuna.net/surveyreports

Jayaprakasha G.K., Jena B.S., Negi P.S., et al. (2002). Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Zeitschrift für Naturforschung C **57:** 828-835

JECFA (1992) FNP 52: Turmeric Oleoresin. Available at: http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-484.pdf

JECFA (1995). Evaluation of certain food additives and naturally occurring toxicants (Fourty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859. Available at: https://apps.who.int/iris/bitstream/handle/10665/37246/WHO_TRS_859.pdf

JECFA (2004). Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 922. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_922.pdf

Joshi J., Ghaisas S., Vaidya A., et al. (2003). Early human safety study of turmeric oil (Curcuma longa oil) administered orally in healthy volunteers. Journal of Association of Physicians of India **51:** 1055-60

Kanai M., Yoshimura K., Asada M. et al. (2011) A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer. Cancer Chemother Pharmacol **68:** 157-164

Kandarkar S.V., Sawant S.S., Ingle AD, et al. (1998) Subchronic oral hepatotoxicity of turmeric in mice-histopathological and ultrastructural studies. Indian J Exp Biol **36:** 675-659

Lao C.D., Ruffin M.T., Normolle D., et al. (2006). Dose escalation of a curcuminoid formulation. Complementary and Alternative Medicine **6:** 10

Latif M.A., Moris T.R., Miah A.M., et al. (1979) Toxicity of shoti (Indian arrowroot: Curcuma zedoaria) for rats and chicks. Br. J. Nutr. **41:** 57-63.

Li S., Yuan W., Deng G., et al. (2011) Chemical composition and product quality control of turmeric (Curcuma longa L.). Faculty Publications. Paper 1. Available at: https://core.ac.uk/download/pdf/72733173.pdf

Liju V.B., Jeena K. & Kuttan R. (2013) Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (Curcuma longa L). Food and Chemical Toxicology **53:** 52-61

Lilja H.S., Hagopian M., Esber H.J., et al. (1983) Report on the subchronic toxicity by dosed fed turmeric oleoresin (C60015) in Fisher 344 rats and B6C3F1 mice. EGG Mason Research Institute, Report No. MRI-NTP 11-83-22. Submitted to WHO by the National Institutes of Health, Research Triangle Park, NC, USA. (cited in: WHO 1987).

Luber R.P., Rentsch C., Lontos S., et al. (2019). Turmeric Induced Liver Injury: A Report of Two Cases. Case Reports in Hepatology doi: 10.1155/2019/6741213.

Lukefahr A.L., McEvoy S., AlfafaraC., et al. (2018) Drug-induced autoimmune hepatitis associated with turmeric dietary supplement use. BMJ Case Rep doi: 10.1136/bcr-2018-224611

National Toxicology Program (NTP). Toxicology and Carcinogenesis Studies of Turmeric Oleoresin (Cas No. 8024-37-1) (Major Component 79%-85% Curcumin, Cas No. 458-37-7) in F344/N Rats and B6C3F1 Mice (Feed Studies). Technical Report Series No. 427, NIH Publication No. 93-3158. U.S. Available at: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr427.pdf

Olojede A.O., Nwokocha C.C., Akinpelu A.O., et al. (2009) Effect of variety, rhizome and seed bed types on yield of turmeric (Curcuma longa L) under a humid tropical agro-ecology. Adv. Bio.Res. **3:** 40-42

Pfeiffer E., Hhle S., Solyom A.S., et al. (2003) Studies on the stability of turmeric constituents. J. Food Engineer. **56:** 257-259

Ravindranath V. & Chandrasekhara N. (1981) Metabolism of curcumin–studies with [^{3}H]curcumin. Toxicology **22:** 337-44

Remya R., Syamkumar S., and Sasikumar B. (2004) Isolation and amplification of DNA from turmeric powder. Br. Food J. **106:** 673-678

Sasikumar B. (2005) Genetic resources of Curcuma: diversity, characterization and utilization. Plant Gen. Resour. **3:** 230-251

SCF (Scientific Committee for Food) (1975). Reports from the Scientific Committee for Food (1st series), opinion expressed 27 June 1975. Accessible via: http://aei.pitt.edu/40814/1/food_1st.pdf

Schiborr C., Kocher A., Behnam D., et al. (2014) The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. Mol. Nutr. Food Res. **58:** 516-527

Sharma R.A., McLelland H.R., Hill K.A., et al. (2001). Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. Clinical Cancer Research **7:** 1894-1900

Sharma R.A., Euden S.A., Platton S.L., et al. (2004) Phase I clinical trial of oral curcumin: Biomarkers of systemic activity and compliance. Clin Cancer Res **10:** 6847-6854

Shoba G., Joy D., Joseph T., et al. (1998) Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. **64:** 353-6

Srimal R.C. & Dhawan B.N. (1973). Pharmacology of deferulolyl methane (Curcumin) a non-steroidal anti-inflammatory agent. Journal of Pharmacy and Pharmacology **25:** 447-452

Stanojević J.S. Stanojević L.P., Cvetković D.J., et al. (2015) Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (Curcuma longa L.). Advanced technologies **4:** 19-25

Stierum R.A., Conesa A., Heihe W., et al. (2008). Transcriptome analysis provides new insights into liver changes induced in the rat upon dietary administration of the food additives butylated hydroxytoluene, curcumin, propylgallate and thiabendazole. Food and Chemical Toxicology **46:** 2616-28

Suhail F.K., Masood U., Sharma A., et al. (2019) Turmeric supplement induced hepatotoxicity: a rare complication of a poorly regulated substance. Clinical Toxicology DOI: 10.1080/15563650.2019.1632882

Tennant D. (2006). Screening of Colour Intakes from Non-Alcoholic Beverages. Report prepared for the Union of European Beverages Associations (UNESDA), December 2006.

Tennant D. (2007). Screening potential intakes of natural food colours. Report provided for the Natural Food Colours Association (NATCOL).

Wahlstrom B. & Blennow G. (1978) A study on the fate of curcumin in the rat. Acta Pharmacol Toxicol **43:** 86-92