GRAS Notice (GRN) No. 460
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

Soni & Associates Inc.

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February 5, 2013

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for Curcumin Preparation (Curcumin C3 Complex®)

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Sabinsa Corporation, through Soni & Associates Inc. as its agent, hereby provides notice of a claim that the food ingredient curcumin preparation (Curcumin C3 Complex®) described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at msoni@soniassociates.net.

Sincerely,
(b) (6)
(b) (6)

Madhu G. Soni, Ph.D., FATS

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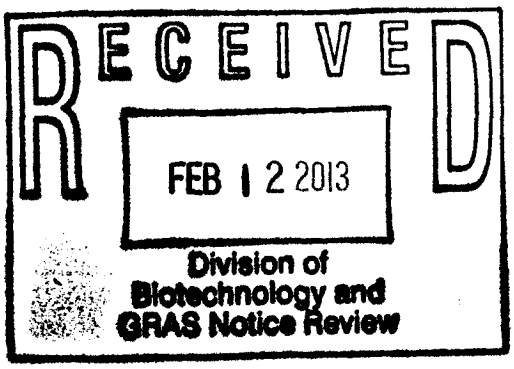

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GRAS NOTIFICATION

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Sabinsa Corporation (the notifier) has determined that curcumin preparation (Curcumin C3 Complex®) derived from the ground rhizomes of *Curcuma longa* L is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use as a food ingredient. Therefore, the use of curcumin preparation (Curcumin C3 Complex®) is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date 2/6/2013

Madhu G. Soni, Ph.D., FATS

Agent for:

Sabinsa Corporation
20 Lake Drive
East Windsor, NJ 08520
USA

000003

B. Name and Address of Notifier:

A. Reza Kamarei, Ph.D.
Vice President, Science and Technology
Sabinsa Corporation
20 Lake Drive
East Windsor, NJ 08520
USA

Tel: (732) 777-1111, Ext. 44
Fax: (732) 777-1443
Email: reza@sabinsa.com

C. Common or Usual Name of the Notified Substance:

The common name of the substance of this notification is curcumin preparation. The preparation is a mixture of curcuminoids (curcumin, bisdemethoxy curcumin and demethoxy curcumin) extracted from the rhizomes of *Curcuma longa* L (turmeric). Generally, the term curcumin is commonly used to represent all the curcuminoids found in the turmeric extract. The trade name of the substance is Curcumin C3 Complex®.

D. Conditions of Intended Use in Food

Curcumin C3 Complex®, a curcumin preparation, is intended for use as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)][1] and as an antioxidant in the following food categories: baked goods; soups; snack foods; imitation dairy products; and seasonings & flavors at use levels up to 20 mg curcumin/serving (reference amounts customarily consumed, 21CFR 101.12). Curcumin preparation is not proposed for uses in foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, as well as it is not intended for use in meat and poultry products that come under USDA jurisdictions. The intended use of Curcumin C3 Complex® in the above mentioned food categories, is estimated to result in a maximum daily intake of 180 mg curcumin/person (3 mg/kg body weight/day for an individual weighing 60 kg). Curcumin C3 Complex® will not be added to food categories that come under USDA jurisdiction.

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of curcumin preparation (Curcumin C3 Complex®) has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. The determination is supported by the opinion of the Expert Panel. A comprehensive search of the scientific literature was also utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for curcumin preparation (Curcumin C3 Complex®). The source material of the curcumin preparation, turmeric has been used as a foodstuff. The use of turmeric, turmeric extract, or turmeric oleoresin in food for human consumption is considered Generally Recognized As Safe (GRAS) by the FDA for use as a coloring and

[1]"Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

flavoring agent in foods. The safety determination of Curcumin C3 Complex® is based on the totality of available evidence.

The safety of Curcumin C3 Complex® is supported by multiple animal and human studies that have been performed with curcumin, turmeric oleoresin and turmeric. Several experimental studies, including subchronic toxicity, chronic toxicity and carcinogenicity, reproduction and developmental toxicity, *in vitro* and *in vivo* genotoxicity and human clinical safety data support the safety in use of curcumin preparation at the intended use levels. Additionally, the safety of curcumin is well established in the literature based on the dietary consumption of foods containing turmeric and turmeric oleoresin. Furthermore, Joint FAO/WHO Expert Committee on Food Additives (JECFA) as well as European Food Safety Authority (EFSA) panel have determined that the available evidence for curcumin supports acceptable daily intake (ADI) of 3 mg curcumin/kg body weight/day. On the basis of scientific procedures [2], Sabinsa Corporation considers the consumption of curcumin preparation (Curcumin C3 Complex®), as a food ingredient to be safe at levels up to 180 mg curcumin/person/day.

F. Availability of Information:

The data and information that forms the basis for this GRAS determination will be provided to Food and Drug Administration upon request or will be available for FDA review and copying at reasonable times at the above mentioned offices of the notifier (Section I, B) or at the offices of:

Madhu G. Soni, PhD, FATS
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32068

Telephone: +1- 772-299-0746
Email: msoni@soniassociates.net

II. Detailed Information About the Identity of the Notified Substance:

Curcumin C3 Complex® is a standardized orange yellow crystalline powder curcumin preparation obtained by solvent extraction of turmeric (*Curcuma longa* L). Curcumin C3 Complex® contains >95% curcuminoids of which between 70 to 80% is curcumin. The other curcuminoids present in the curcumin preparation are bisdemethoxy curcumin (2.5 to 6.5%) and demethoxy curcumin (15 to 25%). Sabinsa Corporation assisted the United States Pharmacopoeia (USP) in the preparation of monographs on curcuminoids and turmeric (Pharmacopeial Forum 33/6, Nov-Dec 2007) and in developing validated analytical methods. Additionally, Sabinsa Corporation provided reference standards for the individual curcuminoids to USP.

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[2] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

A. Chemical name:

Curcumin:	1,7-bis (4'-hydroxy-3'-methoxyphenyl)hepta-1,6-diene-3,5-dione
Bisdemethoxycurcumin:	(1E,6E)-1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione
Demethoxycurcumin:	(1E,6E)-1,6-Heptadiene-3,5-dione,1-(4-hydroxy-3-methoxyphenyl) -7-(4-hydroxyphenyl)

B. Trade Name:

The subject of this notification will be marketed as Curcumin C3 Complex®

C. Chemical Abstract Registry and other Number:

Curcumin: 458-37-7; EINECS No. 207-280-5

D. Chemical Formula:

The empirical formula of curcumin is $C_{21}H_{20}O_6$

E. Structure:

The structural formula of curcumin is presented in Figure II-E.

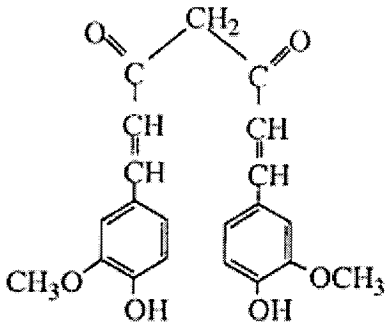

Figure II-E. Chemical Structure of Curcumin

F. Molecular Weight

The molecular weight of curcumin is 368.38 kDa

G. Physical Characteristics

Curcumin C3 Complex® is an orange yellow crystalline powder with a characteristic odor and taste.

H. Typical Composition and Specifications

Typical food grade specifications of curcumin are presented in Tables II-H.1. Analytical data from five manufacturing lots prepared using extraction solvents acetone and ethyl acetate are presented in Appendix I-A and I-B, respectively. General compositional analysis of Curcumin C3 Complex® is presented in Table II-H.2.

Table II-H.1. Specifications of Curcumin C3 Complex® (Curcumin Preparation)

Parameter	**Characteristics (Sabinsa, 2012*)**
Description	Orange yellow crystalline powder
Identification	a) To comply by HPLC; b) To comply by IR Spectrum; c) To comply by UV Absorption
Loss on drying	NMT 2%
Ash content	NMT 1%
Melting range	Melts between 172°C to178°C
Tapped bulk density	Between 0.50g/ml and 0.90g/ml
Loose bulk density	Between 0.30 g/ml and 0.50g/ml
Sieve test (passes through)	
- 20 mesh	NLT 100%
- 40 mesh	NLT 95%
- 80 mesh	NLT 75%
Assay	
Total curcuminoids	NLT 95% on dry basis
Purity of curcuminoids	
Bisdemethoxy curcumin	NLT 2.2% and NMT 6.5%
Demethoxy curcumin	NLT 15% and NMT 22%
Curcumin	NLT 75% and NMT 81%
Heavy metals	
Arsenic	< 1 ppm
Lead	<2 ppm
Cadmium	< 1 ppm
Mercury	< 0.1 ppm
Microbiological assays	
Total plate count	< 5000 cfu/g
Yeast and Mold	< 100 cfu/g
Escherichia coli	Negative (cfu/10 g)
Staphylococcus aureus	Negative (cfu/10 g)
Salmonella	Negative (cfu/10 g)
Pseudomonas aeruginosa	Negative (cfu/10 g)
*Based on information provided by Sabinsa Corporation. NMT = Not more than; NLT = Not less than	

Table II-H-2. Compositional analysis of Curcumin C3 Complex®

Assay	**Typical value (Sabinsa, 2012*)**
Carbohydrate**	95.10%
Fat	3.19%
Protein	0.17%
Moisture	0.51%
Ash	0.98%
Calories	410/100 g
*Based on information provided by Sabinsa Corporation; **Containing carbon, hydrogen and oxygen	

I. Manufacturing process

Curcumin C3 Complex® is manufactured according to current good manufacturing practices (cGMPs) and this process is schematically presented in (Figure II-I). The starting material for the preparation of Curcumin C3 Complex® is dried turmeric rhizomes containing 3-5% curcuminoids. Turmeric is subjected to solvent extraction. The extraction process requires the raw material to be ground into a coarse powder and then pelletized, followed by an acetone or ethyl acetate wash which selectively extracts the coloring matter. The extract is filtered and the acetone or ethyl acetate solution (liquid) is distilled. This process after distillation of the solvent yields an oleoresin with coloring matter content in the region of 25-35% along with volatile oils and other resinous extractives. The oleoresin so obtained is treated with isopropyl alcohol to wash out oily and other resinous matter and extract the curcumin pigment from the oleoresin. This process yields a powdered, purified product, known as curcumin powder, with over 95% curcuminoids. The extraction procedure assures a consistent and high quality Curcumin C3 Complex® product.

J. Manufacturing process diagram

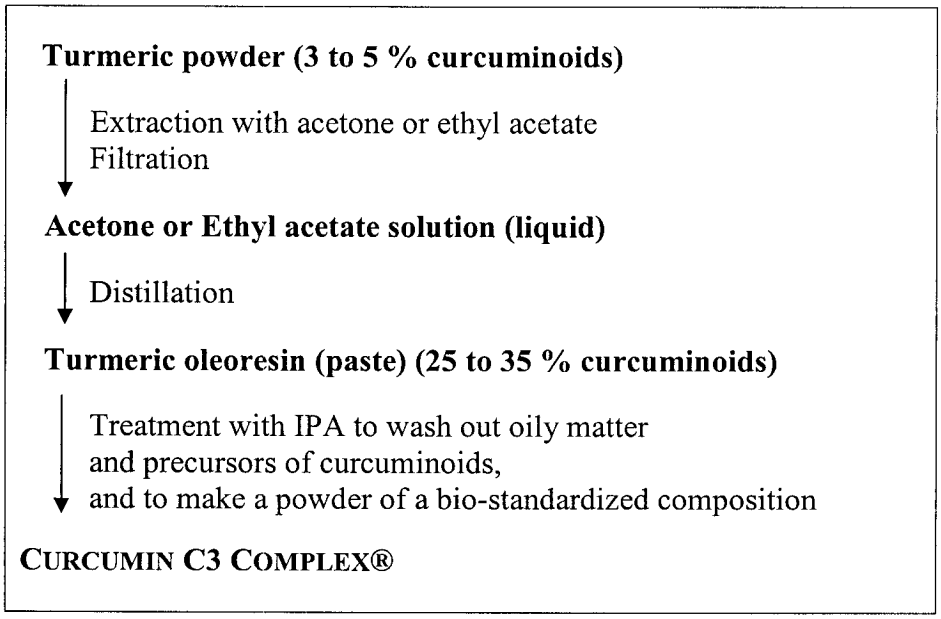

Figure II-I. Manufacturing process of Curcumin C3 complex® (Sabinsa, 2012)

K. Intended Technical Effects

Curcumin preparation is intended for addition to selected foods as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)][3] and as an antioxidant ingredient in the diet. The use of curcumin preparation is intended for the general population at the levels identified in this document for addition to the following food categories: Baked Goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, Sabinsa Corporation does not intend to refer them by the commonly recognized names such as milk, or yogurt.

Use of curcumin preparation in the above described food categories may also incidentally contribute its own color to the product. Its intended use as a flavoring agent and as an antioxidant would thus fall outside the definition of "color additive," in accordance with 21 CFR 70.3(f), "Food ingredients ...which contribute their own natural color when mixed with other foods are not regarded as *color additives*...."

The above exemption from the color additive definition will cover the intended uses of curcumin preparation under the GRAS notification.

III. Summary of the Basis for the Notifier's Determination that Curcumin is GRAS

The determination that Curcumin C3 Complex® is GRAS is based on scientific procedures. A comprehensive search of the scientific literature for safety and toxicity information on curcumin, its oleoresin and turmeric was conducted through January 2013 and was utilized for this assessment. Based on a critical evaluation of the pertinent data and information summarized here and employing scientific procedures, it is determined that the addition of curcumin preparation to the selected foods described in this notice and at use levels of 20 mg/serving (in accordance with established reference amounts customarily consumed, 21 CFR 101.12) meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is GRAS under the conditions of intended use as specified herein.

In coming to this decision that curcumin preparation is GRAS, Sabinsa Corporation relied upon the conclusions that neither curcumin nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

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[3]"Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

IV. Basis for a Conclusion that Curcumin is GRAS for its Intended Use.

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to determine the safety of Curcumin C3 Complex® used as a food ingredient to provide consumers with a source of curcumin in their diets. Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of curcumin preparation (Curcumin C3 Complex®) in baked goods; soups; snack foods; imitation dairy products; and seasonings & flavors at use levels up to 20 mg curcumin/serving (reference amounts customarily consumed, 21CFR 101.12) when not otherwise precluded by a Standard of Identity as described here and resulting in the 90th percentile all-user estimated intake of 180 mg curcumin/person/day is GRAS. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion (see attached Expert Panel Statement).

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CURCUMIN (CURCUMIN C3 COMPLEX®) AS A FOOD INGREDIENT

Prepared by
Soni & Associates Inc.
749 46th Square
Vero Beach, FL 32968

Prepared for
Sabinsa Corporation
20 Lake Drive
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Panel Members

John A. Thomas, Ph.D., F.A.T.S.
Stanley M. Tarka, Jr., Ph.D.
Madhusudan G. Soni, Ph.D., F.A.T.S.

February 2013

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CURCUMIN (CURCUMIN C3 COMPLEX®) AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CURCUMIN (CURCUMIN C3 COMPLEX®) AS A FOOD INGREDIENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Soni & Associates Inc., at the request of Sabinsa Corporation, USA, to determine the Generally Recognized As Safe (GRAS) status of curcumin (Curcumin C3 Complex®) as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)][2] and as an antioxidant in selected food products (Baked goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors) at use levels of up to 20 mg curcumin/serving (reference amounts customarily consumed, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on curcumin and turmeric was conducted through December 2012 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Sabinsa Corporation and other information deemed appropriate or necessary. Sabinsa Corporation assures that all unpublished information in its possession and relevant to the subject of this determination has been provided to Soni & Associates Inc. and has been summarized in this GRAS monograph. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

1.1. Background

Curcumin, a diferuloylmethane and active ingredient of the dietary spice turmeric, is obtained from the ground rhizomes of *Curcuma longa* L. The turmeric plant is a perennial herb belonging to the ginger family that is cultivated extensively in India and Southeast Asia. The term curcumin is commonly used to represent all the curcuminoids found in the extract. Curcumin is the characteristic yellow coloring component of turmeric. Curcumin has also been reported to possess a wide range of therapeutic uses in traditional Indian (Ayurveda, Unani, and Siddha) systems of health care. It was first isolated in 1815 and obtained in crystalline form in 1870 (Shishodia et al., 2008; Daybe, 1870). Curcumin is the most biologically active curcuminoid of turmeric and comprises 2-5% of the spice. Curcumin has a variety of uses including as a dietary spice, as a coloring agent in foods and textiles, and as therapeutic agent for numerous health conditions. Curcumin has been consumed for centuries.

Ayurveda (*Ayur* = long life; *veda* = knowledge), the ancient texts of the Indian traditional health care system, describe the use of curcumin for a wide variety of inflammatory conditions including sprains and swellings caused by injury, wound healing, and abdominal problems (Ammon and Wahl, 1991). Texts on traditional medicine in China describe ingestion of curcumin for relief from abdominal pain. It has been suggested that most of the effects associated with curcumin may be based on its ability to suppress inflammation (Sikora et al., 2010). First

[1]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

[2]"Flavor enhancers": Substances added to supplement, enhance, or modify the original taste and/or aroma of a food, without imparting a characteristic taste or aroma of its own.

demonstrated to have antibacterial activity in 1949, curcumin has since been shown to possess anti-inflammatory, anti-oxidant, pro-apoptotic, chemopreventive, chemotherapeutic, antiproliferative, wound healing, antinociceptive, antiparasitic and antimalarial properties as well (Gupta et al., 2012). Extensive research over the last half century has revealed several important biological effects of curcumin such as antioxidant, anti-inflammatory, anti-cancer, and anti-atherogenic. Given the beneficial properties of curcumin, Sabinsa Corporation intends to market a well-characterized powder extract of uniform milled curcumin, under the name Curcumin C3 Complex®, as a food ingredient.

1.2. Description

The subject of this GRAS determination is Curcumin C3 Complex®, a standardized extract prepared by solvent extraction (using acetone or ethyl acetate) of turmeric (dried rhizome of *C. longa* L). The extract contains curcuminoids (curcumin, bisdemethoxy curcumin and demethoxy curcumin). As referenced in the published literature and in this document, the term curcumin also refers to all the curcuminoids found in the extract. Curcumin (Figure 1) is chemically known as 1,7-bis (4'-hydroxy-3'-methoxyphenyl)-1,6-heptadiene-3,5-dione. Curcumin C3 Complex® is orange yellow crystalline powder with a characteristic odor and taste. General descriptive characteristics of Curcumin C3 Complex® are summarized in Table 1.

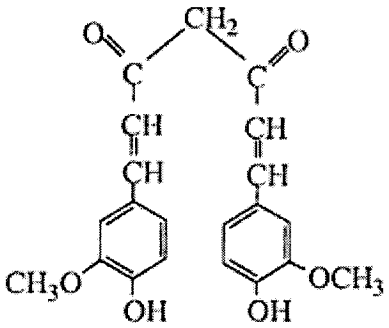

Figure 1. Chemical structure of curcumin

Table 1. General descriptive characteristics of Curcumin C3 Complex®

Parameter	Description (Sabinsa, 2012)*
Plant Source	*Curcuma longa*
Part used	Rhizomes and roots
CAS No.	458-37-7
EINECS No.	207-280-5
Synonyms/other names	Curcuma Longa extract; Curcumin; Demethoxycurcumin; Bisdemethoxy curcumin
Appearance	Crystalline powder
Solubility	Insoluble in water, soluble in ethanol
Color	Orange yellow
Odor	Characteristic
Taste	Characteristic
Molecular weight	368.38 (for curcumin).
Chemical formula	$C_{21}H_{20}O_6$
Storage	Room temperature

*Based on information provided by Sabinsa Corporation

1.3. Specifications and Identity

Typical food grade specifications of Curcumin C3 Complex® from Sabinsa Corporation are presented in Table 2. The curcuminoid content of Curcumin C3 Complex® is >95%. Among the curcuminoids, the level of curcumin in Curcumin C3 Complex® ranges from 70-80%. Analytical results from five non-consecutive lots manufactured using the extraction solvents acetone (Appendix I-A) and for three lots using ethyl acetate (Appendix I-B) demonstrate that Curcumin C3 Complex® meets the standard specifications. Curcumin C3 Complex® is slightly soluble in alcohol, acetone and glacial acetic acid and is insoluble in water. Recently, Sabinsa Corporation assisted the United States Pharmacopoeia (USP) in the preparation of monographs on curcuminoids and turmeric (Pharmacopeial Forum 33/6, Nov-Dec 2007) and in developing validated analytical methods. Additionally, Sabinsa Corporation provided reference standards for the individual curcuminoids to USP.

The residual solvent levels for acetone and isopropyl alcohol (Appendix I-A) that are used in the manufacturing of Curcumin C3 Complex® are below the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Code of Federal Register (CFR) regulations cited limits for these solvents. The alternative solvent, ethyl acetate, employed in the extraction of curcumin is permitted under regulation 21 CFR 172.859 in the preparation of sucrose esters. According to this regulation, the specification for ethyl acetate in sucrose esters is established at < 350 ppm. Additionally, as per 21 CFR 172.372 residual level of ethyl acetate (< 500 ppm) is permitted as a processing aid in the nutrient N-acetyl-L-methionine. Ethyl acetate is also permitted as a secondary direct food additive in accordance with current good manufacturing practice (cGMP) as a solvent in the decaffeination of coffee and tea (21 CFR 173.228). The residual levels of ethyl acetate in Curcumin C3 Complex® from three manufacturing lots are below 50 ppm (Appendix I-B). The trend analysis also confirms that the Sabinsa Corporation's Curcumin C3 Complex® complies with International Conference on Harmonization (ICH) and USP guidelines for residual solvents (Appendix I).

Table 2. Specifications of Curcumin C3 Complex®

Parameter	Characteristics (Sabinsa, 2012*)
Description	Orange yellow crystalline powder
Identification	a) To comply by HPLC; b) To comply by IR Spectrum; c) To comply by UV Absorption
Loss on drying	NMT 2%
Ash content	NMT 1%
Melting range	Melts between 172°C to178°C
Tapped bulk density	Between 0.50g/ml and 0.90g/ml
Loose bulk density	Between 0.30 g/ml and 0.50g/ml
Sieve test (passes through)	
- 20 mesh	NLT 100%
- 40 mesh	NLT 95%
- 80 mesh	NLT 75%
Assay	
Total curcuminoids	NLT 95% on dry basis
Purity of curcuminoids	
Bisdemethoxy curcumin	NLT 2.2% and NMT 6.5%
Demethoxy curcumin	NLT 15% and NMT 22%
Curcumin	NLT 75% and NMT 81%
Heavy metals	
Arsenic	< 1 ppm
Lead	< 2 ppm
Cadmium	< 1 ppm
Mercury	< 0.1 ppm
Microbiological assays	
Total plate count	< 5000 cfu/g
Yeast and Mold	< 100 cfu/g
Escherichia coli	Negative (cfu/10 g)
Staphylococcus aureus	Negative (cfu/10 g)
Salmonella	Negative (cfu/10 g)
Pseudomonas aeruginosa	Negative (cfu/10 g)
*Based on information provided by Sabinsa Corporation. NMT = Not more than; NLT = Not less than	

General compositional analysis of Curcumin C3 Complex® is presented in Table 3.

Table 3. Compositional analysis of Curcumin C3 Complex®

Assay	Typical value (Sabinsa, 2012*)
Carbohydrate**	95.10%
Fat	3.19%
Protein	0.17%
Moisture	0.51%
Ash	0.98%
Calories	410/100 g
*Based on information provided by Sabinsa Corporation; **Containing carbon, hydrogen and oxygen	

1.4. Manufacturing Process

Curcumin C3 Complex® is manufactured according to current good manufacturing practices (cGMPs) and this process is schematically presented in Figure 2. The starting material for the preparation of Curcumin C3 Complex® is dried turmeric rhizomes containing 3-5% curcuminoids. The extraction process requires the raw material to be ground into a coarse powder and then pelletized, followed by an acetone or ethyl acetate wash which selectively extracts the coloring matter. The extract is filtered and the acetone or ethyl acetate solution (liquid) is distilled. The resulting oleoresin paste contains 25-35% curcuminoid along with volatile oils and other resinous extractives. The oleoresin is treated with isopropyl alcohol to wash out oily and other resinous matter and precursors of curcuminoids present as a result of the extraction. This process yields a powdered, purified product, known as curcumin powder, with over 95% curcuminoids. The extraction procedure assures a consistent and high quality Curcumin C3 Complex® product.

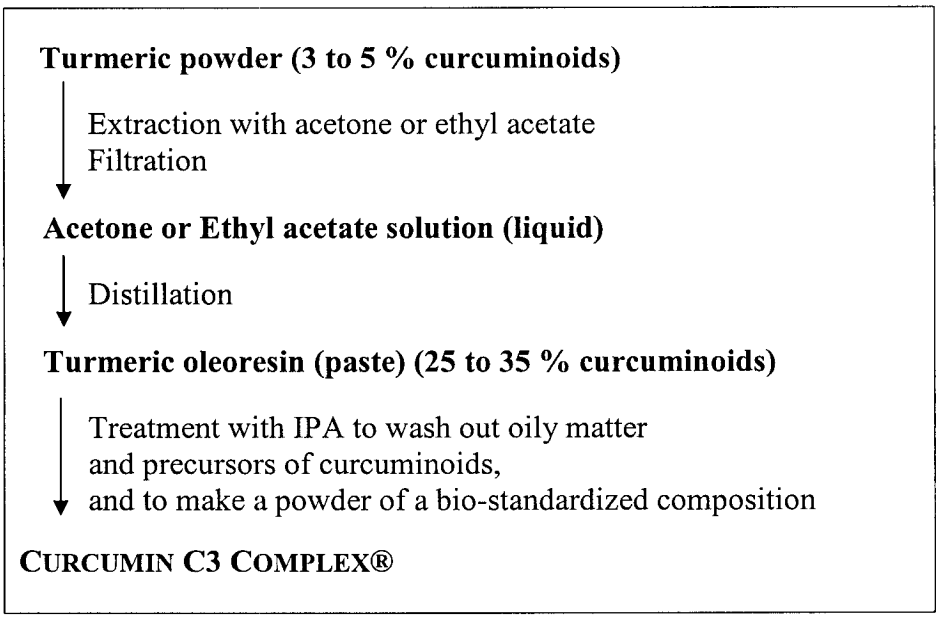

Figure 2. Manufacturing process of Curcumin C3 complex® (Sabinsa, 2012). The residual solvent levels of acetone or ethyl acetate complies with USP guidelines for residual solvents.

1.5. Current Uses

Traditionally, turmeric has been used as a foodstuff, a cosmetic, and for therapeutic purposes. As a spice, turmeric is used in curry to provide its distinctive yellow color and flavor. It is also used (where permitted by food regulations) as a coloring agent in cheese, butter, and other foods (Govindarajan, 1980; Goel et al., 2008). Curry powder which may contain 10-30% curcumin (Govindarajan, 1980) has also been reported to be added to gelatins pudding (0.05 ppm), soups (30-50 ppm), meats (200 ppm), and pickles (690 ppm). Curcumin is widely used in foods as a flavoring agent and color additive (21 CFR§73.600 and §73.615). The major components in all turmeric oleoresins are curcuminoids, primarily curcumin. The advantage of using turmeric oleoresin as a food additive rather than turmeric is that the organic extraction procedure removes microbial contaminants that might be found in ground powder (Govindarajan, 1980). The United States is the largest consumer of turmeric oleoresins. The oleoresin is used as

a food color and imparts a characteristic mild spicy aroma to products such as mustard, pickles and relishes. The oleoresin (FEMA 3087) is added to condiments (640 ppm), meats (20-100 ppm) and pickles (200 ppm). Taken together, this demonstrates that turmeric, turmeric oleoresin and curcumin have been widely used as a spice and as a coloring in a variety of different foods.

The Draft Codex General Standard for Food Additives provides an extensive list of foods in which curcumin is used (Stankovic, 2004). Curcumin is listed for use in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and eggs products, spices, soups, sauces and protein products, foodstuffs intended for particular nutritional uses, beverages, ready-to-eat savories and composite foods. Depending on the food category, use levels of curcumin range from 5 to 500 mg/kg. In a recent amendment, the Codex General Standard for processed cheese preparations, cheese food and cheese spread permits use of curcumin as a coloring agent at GMP levels (Codex, 2008).

Curcumin is used as a fragrance in soaps, detergents, creams, lotions and perfumes. Both turmeric and curcumin are also used as herbal supplements or as ingredients in multiple supplement products. These products are available in capsules, solutions, and tablets (Hendler and Rorvik, 2001). As dietary supplements, these products are regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994). The available information suggests that the principal constituent of Curcumin C3 Complex® has a long history of human consumption. As a dietary supplement the recommended or suggested use of Curcumin C3 Complex® is 1.5 g/day (based on internet searches).

1.6. Regulatory Status

Over the years, the JECFA has frequently evaluated the use of curcumin as a food additive. Most recently (JECFA, 2004), the JECFA established an acceptable daily intake (ADI) of curcumin of 0-3 mg/kg body weight. JECFA has not allocated ADI values to turmeric or turmeric oleoresin. JECFA often declines to allocate ADI's for food additives and for substances regarded as a food and devoid of adverse effects such that the intake of substances arising from its use levels (as determined by good manufacturing practice) and its acceptable background in food does not represent a hazard to health (Groten et al., 2000). The committee also noted that turmeric is often regarded as a food rather than as a food additive (JECFA, 2001, 2003). Similar to JECFA, recently the European Food Safety Authority (EFSA) panel also concluded that the available evidence supports an ADI of 3 mg/kg body weight (bw)/day for curcumin (EFSA, 2010).

Turmeric and turmeric oleoresin are also on the FDA's list of color additives approved for use in human foods (21 CFR 73.600, 73.615). The FDA defines turmeric oleoresin as the combination of flavor and color principles obtained from turmeric by solvent extraction. As mentioned earlier, the major component of the oleoresin is curcumin. The use of turmeric, turmeric extract, or turmeric oleoresin in food for human consumption is considered Generally Recognized As Safe (GRAS) by the FDA for use as a coloring and flavoring agent in foods when used in accordance with good manufacturing practice (21 CFR 182.10, 21 CFR 182.20). Use of turmeric is also listed under 21 CFR 169.140, 21 CFR 169.150 as a food dressing and flavoring agent in mayonnaise and salad dressing, respectively. Turmeric, turmeric extract and turmeric oleoresin are also considered as GRAS by the Flavoring and Extract Manufacturers' Association (FEMA) (Hall and Oser, 1965).

1.7. Technical Effects

The intended uses of Curcumin C3 Complex® are for addition as a food ingredient, flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] and as an antioxidant to food. Because of its characteristic flavor enhancing effects, use of Curcumin C3 Complex® influences the sensory perception of food. While the use of the extract in foods may also impart a color to food products, the intended use of Curcumin C3 Complex® would fall outside the definition of "color additive" for the following reasons: the extract is solely added for its flavoring and thus may constitute an "unimportant color" [21 CFR 70.3(g)] and does not relate to any use of the ingredient as a color additive [21 CFR 70.3(f)].

1.8. Estimated Intake from Natural Presence in Food and Added Uses

The average daily intake of curcumin through ingestion of turmeric in India was reported to range from 0.4 to 1.5 mg/kg body weight/day (24 to 90 mg/kg/day for an individual weighing 60 kg) (Srinivasan and Satyanarayana, 1988). Similarly, Shah et al. (1999) reported the average daily intake of curcumin in the diet in India to be approximately 60 to 100 mg curcumin/day based on an average turmeric intake of 2 to 2.5 g/day. Commandeur and Vermeulen (1996) estimated the average daily intake of curcumin by adults in India to range from 80 to 200 mg/day. The average daily intake of curcumin in France was reported to be 1 mg/kg bw/day, with a theoretical maximum daily intake of 4.5 mg/kg bw/day (Verger et al., 1998). FEMA reports an estimated individual daily intake of 0.203 mg/kg bw/day and a Possible Average Daily Intake (PADI) of 62.142 mg.

1.9. Intended Use Levels and Food Categories

Sabinsa Corporation intends to use Curcumin C3 Complex®, as a flavoring agent at use levels of up to 20 mg/serving in Baked goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors (Table 4). Although some foods with standards of identity are included in the list of foods, at present, the use of Curcumin C3 Complex® is intended for foods without a standard of identity. Additionally, foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, as well as meat and poultry products that come under USDA jurisdictions are excluded from the list of intended food uses of the subject curcumin preparation (Curcumin C3 Complex®).

1.9.1. Estimated Daily Intake from the Intended Uses

1.9.1.1. Use of MRCA exposure data

Estimates of possible daily intake of curcumin from the "maximum" intended use levels of Curcumin C3 Complex® have been determined using the mean consumption estimates of designated food categories based on Market Research Corporation of America (MRCA) mean frequency of eating and USDA mean portion size of 34 general food categories data (MRCA, 1965). In addition to intake surveys by USDA, marketing research groups such as MRCA and the National Purchase Diary (NPD) have also surveyed the food consumption patterns of individuals and households. The primary purpose of these surveys is to determine the nutritional adequacy of diets rather than the safety of food with respect to additives or contaminants. However, these surveys are frequently used to assess exposure to additives and contaminants. Under an FDA contract, the Federation of American Societies for Experimental Biology (FASEB, 1988) reviewed the theory behind the calculation of exposure estimates from different

surveys including the MRCA. The FDA's Office of Premarket Approval (OPA) has historically relied on MRCA survey to determine consumption estimates.

Although the MRCA method was developed in 1965, it is still accepted by the FDA in determining the possible average daily intake of food ingredients (DiNovi and Kuznesof, 1995). The conservatism of this determination method assumes that the maximum amount of substance is added to the entire food category, not just the food product within that category. Using MRCA estimated mean intakes of the food categories (Baked goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors) for which Curcumin C3 Complex® is proposed to be added, the possible maximum daily intake of Curcumin C3 Complex® from each of the categories is summarized in Table 4.

Table 4. Proposed Use Levels and Possible Daily Intake of Curcumin Based on MRCA Data[1]

Food category	Mean consumption of food product (g/day)	Use levels/ serving (mg)	Approximate serving size[2](g)	Mean Daily intake (mg/ person)	High (90th %) Daily intake (mg/person)
Baked Goods	137.2	20	55	49.89	99.78
Snack Foods	1.30	20	30	0.87	1.73
Soups	31.7	20	245	2.59	5.18
Imitation Dairy Products	0.9	20	225	0.08	0.16
Seasonings & Flavors	0.01	20	0.5	0.40	0.80
			Total (mg/person/day)	**53.85**	**107.55**

[1]The daily intake calculations are based on MRCA (1965) data on mean frequency of eating and USDA report on mean portion size. [2]Serving size is based on Reference Amounts Customarily Consumed per Eating Occasion (21 CFR 101.12) and other related information.

Analysis of the results of projected maximum consumption and thus exposure revealed that the intended uses of Curcumin C3 Complex® in the specified food categories will result in a mean estimated daily intake of.53.85 mg of Curcumin C3 Complex®/person. In order to estimate the 90th percentile consumption of Curcumin C3 Complex®, the corresponding mean total intake value from all food categories was multiplied by two on the grounds that the 90th percentile consumption rarely exceeds the mean by more than a factor of two. These assumptions and the analysis above indicate that the mean and 90th percentile estimated daily intake of the Curcumin C3 Complex® from its intended food uses is 53.85 and 107.55 mg/person/day, respectively.

1.9.1.2. Use of USDA Data

Based on USDA CSFII surveys (Smiciklas-Wright et al., 2002) for quantities of foods consumed daily, the mean and 90th percentile consumption of Curcumin C3 Complex® from the proposed uses in Baked goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors were determined (Table 5). The CSFII data provides intake of several different types of baked goods. In Table 5, values for biscuits are included. Similarly, for imitation dairy products, yogurt is used as a representative category. The intended use of Curcumin C3 Complex® at levels of 20 mg *per* serving will result in mean and 90th percentile intake of 97.44 and 180.25 mg/person/day, respectively.

Table 5. Intended Use Levels (12..5 mg/serving) and Possible Daily Intake of curcumin Based on USDA Data[1]

Food category	Consumption of food product (g/day)		Use levels/ serving (mg)	Serving size; RACC (g)	Daily intake by adult (mg/person)	
	Mean	90th %			Mean	90th %
Baked Goods[2]	64	118	20	55	23.27	42.91
Soups	398	697	20	245	32.49	56.90
Snack Foods	41	84	20	30	27.33	56.00
Imitation Dairy Products[3]	157	266	20	225	13.95	23.64
Seasonings & Flavors	0.01*	0.02*	20	0.5	0.40	0.80
				Total (mg/person/day)	**97.44**	**180.25**

[1]The daily intake calculations are based on USDA data (CSFII) and mean portion size; [2]Biscuits intake is used to represent baked good. [3]Includes yogurt as representative for this category. *As CSFII did not report consumption, MRCA data was used. Serving size is based on Reference Amounts Customarily Consumed per Eating Occasion (21 CFR 101.12) and other related information.

1.9.2. Consumption Summary

The estimated intake of curcumin from its natural presence in turmeric ranged from 24-200 mg/day. The high intake of curcumin is likely to be in countries like India where turmeric is commonly consumed. Based on MRCA data, the intended use of Curcumin C3 Complex® (20 mg/serving) in food categories such as in Baked goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors will result in mean and 90th percentile estimated daily intake of 53.85 and 107.55 mg/person/day, respectively. Based on the USDA CSFII database, the intended use of Curcumin C3 Complex® will result in mean and 90th percentile intakes of 97.44 and 180.25 mg/person/day, respectively. For safety assessment purposes, the high intake of 180 mg Curcumin C3 Complex®/person/day (3 mg/kg bw/day) is considered as appropriate.

2. TOXICOLOGY

The safety of curcumin is supported by several lines of evidence including multiple human clinical trials, as well as by a variety of animal and *in vitro* experimental studies that further corroborate the human observations. Because of its known health benefits and historical use, there has been considerable effort to elucidate the biological role of curcumin, the active principle of turmeric, in the human body. As a result, the literature is full of information on turmeric and curcumin. In the published literature, over 5000 preclinical *in vitro* and *in vivo* studies with curcumin and turmeric have appeared. Additionally over 40 different completed clinical trials and ~30 ongoing Phase I and II efficacy-related trials can be found in the literature. Relevant biological and toxicological studies on curcumin and turmeric are included in the following section. The safety data from animal studies is extensive and sometimes equivocal. Efforts have been made to present both the data supporting the safety as well as any data on the toxicity of curcumin. The safety and toxicity data of turmeric and its extract are also extensive and thus, only relevant studies are briefly discussed in the following sections for the sake of completeness and to support the safety of curcumin.

2.1. Absorption, Metabolism and Excretion

Pharmacokinetic studies of curcumin are summarized in Table 6. Preclinical data from both studies using animal models and clinical studies performed with human volunteers and cancer patients have all demonstrated low systemic bioavailability of curcumin following oral administration (Shehzad et al., 2010). Efficient first-pass metabolism and some degree of intestinal biotransformation might explain its poor systemic bioavailability when administered via the oral route.

Table 6. Summary of pharmacokinetic studies of curcumin and turmeric extract

Reference	Species	Route	Dose	Findings
Animal studies				
Wahlstom and Blennow (1978)	Rats (Sprague-Dawley)	Oral	1 g/kg	75% excreted in feces; negligible in urine; poorly absorbed in gut; no toxicity at 5 g/kg
Wahlstom and Blennow (1978)	Rats (Sprague-Dawley)	IV	-	Transported in bile; major part metabolized
Holder et al. (1978)	Rats (Sprague-Dawley)	Oral; IV; IP	*	Mostly fecal excretion; excreted in bile; major biliary metabolite tetrahydro- and hexahydro-curcumin glucuronides
Ravindranath and Chandrasekhara (1982)	Rats (Wistar)	Oral	400 mg	60% absorbed; none in urine, conjugated glucuronides and sulfate; none in heart blood; < 5µg/ml in portal blood; negligible in liver/kidney (< 20µg/tissue) for 24 hrs; at 24 hrs 38% in lower part of gut
Shoba et al. (1998)	Rats (Wistar)	Oral	2 g/kg	Low serum levels; piperine increased bioavailability by 154%
Sharma et al. (2001a)	Rats	Oral	2% of diet	Plasma levels 12 nM; detected in liver, colon; more in plasma, less in colon
Ireson et al. (2001)	Rats	Oral	500 mg/kg	Detected in plasma; biotransformed to curcumin glucuronide and sulfate
Ireson et al. (2001)	Rats	IV	40 mg/kg	Disappeared from plasma in 1 hr
Pan et al. (1999)	Mice	IP	100 mg/kg	2.25 µg/ml in plasma in first 15 min; at 1 hr-intestine, spleen, liver, kidney-177, 26, 27, 8 µg/kg; biotransformed from dihydrocurcumin to trihydrocurcumin and then to monoglucuronide conjugates
Perkins et al. (2002)	Mice	IP	100 mg/kg*	39-240 nmol/g tissue small intestine
Human studies				
Shoba et al. (1998)	Human	Oral	2 g	Serum levels non-detectable; piperine increased bioavailability by 2000%
Lal et al. (1999)	Human	Oral	375 mg x 3/day	Well tolerated for 12 weeks
Lal et al. (2000)	Human	Oral	375 mg x 3/day	Well tolerated for 6-22 months
Cheng et al. (2001)	Human	Oral	1 – 12 g/day	Serum levels peaked at 1-2 hrs and declined at 12 hrs
Sharma et al. (2001b)	Human	Oral	36-180 mg/day	None in blood or urine; most in feces; 59% decrease in lymphatic GST after 14 days
Gota et al. (2010)	Human	Oral	650 mg, single dose	C_{max} for solid lipid curcumin particle was found to be 22 ng/ml, while for generic curcumin it was <1 ng/ml

Reference	Species	Route	Dose	Findings
Kanai et al. (2012)	Human	Oral	150 and 210 mg, single dose	C_{max} for nanoparticle curcumin at 150 and 210 mg was 189 and 275 ng/ml; AUC for 24 hour was 2,649 and 3,649 ng/ml × hour; $t_{1/2}$ was 9.7 and 13 hour, respectively.
*Combined with [^{3}H]-curcumin; IV = intravenous; IP = intraperitoneal; GST = glutathione s-transferase				

2.1.1. Animal Studies

Pharmacokinetics of curcumin has been investigated in animals *via* a variety of routes such as systemic, intravenous and oral routes of administration (Table 6). Wahlstom and Blennow (1978) reported that oral administration of curcumin to Sprague-Dawley rats at dose level of 1 g/kg body weight resulted in very low or undetectable amounts of curcumin in the blood, urine and bile. The majority of the dose (65 to 85%) was excreted in the feces. Fecal excretion was highest during the first 48 hours, and only 1 to 3% of the administered curcumin was excreted between 48 and 72 hours. In another study, Ravindranath and Chandrasekhara (1982) reported that following oral administration of bolus doses of 10, 80, or 400 mg [^{3}H]-curcumin to male Wistar rats, no curcumin was detected in urine. The percentage of curcumin absorbed (60% to 66%) remained constant over the range of doses studied, and curcumin was detected in the blood, liver, and kidney. At doses of 10 and 80 mg, the bulk of the curcumin was excreted in the feces (60% to 90%) within 3 days, while at the 400 mg dose, excretion in the feces was more prolonged, occurring over a 12-day period. The investigators suggested that the prolonged excretion pattern was indicative of enterohepatic circulation. This is supported by another study by Holder et al. (1978), where glucuronide conjugates of curcumin, were found in the bile. Ravindranath and Chandrasekhara (1982) also indicated that the differences between their findings and those of Wahlstom and Blennow (1978) might have been attributed to strain differences or the use of a different vehicle and dose. Additionally in the Wahlstom and Blennow (1978) study curcumin was measured by spectrofluorimetric method and this method may not have been able to detect conjugates of curcumin occurring in blood and urine.

Holder et al. (1978) investigated metabolism and excretion of [^{3}H]-curcumin following oral, intraperitoneal and intravenous administration to male Sprague Dawley rats. Following oral administration of a 0.6 mg curcumin dose, 89.4 and 6.3% of the dose was detected in the feces and urine, respectively, within 72 hours. After intraperitoneal administration, 80% of the label was excreted in the feces and ~10% appeared in urine within 72 hours. Following an intravenous administration, the bile from a cannulated rat was found to contain 85% of the label. The primary metabolites identified in the bile were glucuronides of tetrahydrocurcumin and hexahydrocurcumin. Following intravenous and intraperitoneal administration of curcumin, active transport by the bile and extensive metabolism by the liver were demonstrated.

The bioavailability of curcumin following oral administration was estimated to be approximately 65%. Commandeur and Vermeulen (1996) reported that curcumin inhibits cytochrome P450 isoenzyme 1A1 (CYP 1A1) and is metabolized and then conjugated primarily by glucuronidation and excreted in the feces. In an *in vitro* study, where curcumin was incubated with both hepatocytes and a microsomal suspension, curcumin was reported to be rapidly metabolized (90% metabolized within 30 minutes). *In vivo* studies also suggest rapid metabolism of curcumin. In the Holder et al. (1978) study, 85% of the curcumin was recovered in the bile after 6 hours following administration of labeled curcumin to cannulated rats by intravenous

injection. In this study, the major metabolites noted were the glucuronides of tetrahydrocurcumin and hexahydrocurcumin, with dihydroferulic acid and ferulic acid present as minor metabolites.

In another study, Asai and Miyazawa (2000) studied the absorption and metabolism of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) following oral administration to 7-week old male Sprague- Dawley rats. The predominant plasma metabolites were noted to be glucuronides and glucuronide/sulfates of curcuminoids. Plasma concentrations of conjugated curcuminoids reached a maximum one-hour following administration. The conjugative enzyme activities for glucuronidation and sulfation of curcumin were found in the liver, kidneys and intestinal mucosa. These investigators concluded that orally administered curcuminoids are absorbed from the gastrointestinal tract and are present in the general blood circulation after largely being metabolized to glucuronide and glucuronide/sulfate conjugates.

Pan et al. (1999) investigated the pharmacokinetics of curcumin in mice following intraperitoneal injection. Following the administration of curcumin at a dose level of 0.1 g/kg body weight, approximately 2.25 μg/ml of the curcumin appeared in the plasma in the first 15 min. At one hour after administration, the levels of curcumin in the intestines, spleen, liver, and kidneys were 177, 26, 27, and 8 μg/g, respectively, with only traces (0.41 μg/g) detected in the brain. Curcumin was first biotransformed to metabolites dihydrocurcumin and tetrahydrocurcumin that were subsequently converted to monoglucuronide conjugates. The results of this study along with subsequent investigations from this group (Lin et al., 2001) suggest that curcumin glucuronide, dihydrocurcumin-glucuronide, tetrahydrocurcumin-glucuronide, and tetrahydrocurcumin are the major metabolites of curcumin in mice.

Ireson et al. (2002) examined curcumin metabolism in subcellular fractions of human and rat intestinal tissue, compared it with metabolism in the corresponding hepatic fractions, and studied curcumin metabolism *in situ* in intact rat intestinal sacs. These investigators reported that extensive conjugation of curcumin occurs in the gastrointestinal tract of humans, and that there is an increased metabolism of curcumin in human than observed in rat intestinal tissue. Curcumin conjugation was less extensive in hepatic fractions from humans than in rats. In a review article, Chainani-Wu (2003) indicated that the findings from Ireson et al. (2002) may explain hepatotoxicity noted in some rodent studies and increased susceptibility noted in rodents (see sections 2.3 and 2.4) (NTP, 1993).

2.1.2. Human Studies

In humans (n=10) receiving a single oral dose of 2 g curcumin, serum levels were either undetectable or very low (Shoba et al., 1998). However, concurrent administration of curcumin with 20 mg piperine, a known inhibitor of hepatic and intestinal glucuronide conjugation, resulted in very high serum levels of curcumin from 0.25 to 1 hour post-dosing, and bioavailability of curcumin was increased by 2000%. No toxicity was observed in the 10 subjects who participated in this study (Shoba et al., 1998). In a dose escalation study (Sharma et al., 2001b), fifteen patients with advanced colorectal cancer received an extract of turmeric (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. Neither curcumin nor its metabolites (glucuronide or sulphate conjugates, or hexahydrocurcumin or hexahydrocurcuminol) were detected in blood or urine, but curcumin was recovered from feces. These investigators concluded that curcumin has low oral bioavailability in humans and may undergo intestinal metabolism (Sharma et al., 2001b). According to Sharma et al. (2001b), no dose-limiting toxicity

was found following oral administration of 2200 mg of turmeric extract containing 180 mg of curcumin for a period of four months.

In a clinical trial cited in a review article (Johnson and Mukhtar, 2007), the presence of curcumin and its metabolites in hepatic tissue and portal blood was investigated. In this study, twelve patients received 450, 1800 or 3600 mg of curcumin capsules for seven days prior to surgery. Only 3 of the 12 subjects receiving 3600 mg/day of curcumin had levels at the threshold of detection (~3 nmole). Curcumin, curcumin sulfate, and curcumin glucuronide were not present in bile or liver tissue in any patient. Two reductive forms of curcumin (hexahydrocurcumin and hexahydrocurcuminol) were seen in one patient. Garcea et al. (2005) studied curcumin levels in the colorectum and determined the pharmacodynamics in 12 patients with confirmed colorectal cancer. Patients were assigned to 450, 1800 or 3600 mg of curcumin/day for seven days prior to surgery. Detectable curcumin levels were seen in the serum of only 1 patient (3600 mg). Every patient had detectable curcumin levels in normal and malignant colorectal tissue ranging from 7 to 20 nmol/g tissue. Curcumin levels were the highest in normal tissue of the cecum and ascending colon as opposed to the transverse, splenic flexure, and descending colon suggesting a local effect.

Vareed et al. (2008) examined the pharmacokinetics of curcumin (Curcumin C3 Complex) in healthy human volunteers at 0.25 to 72 hours following a single oral dose of 10 g (n = 6) or 12 g (n = 6). Serum samples were assayed for free curcumin, and for its glucuronide and sulfate conjugates. The data were fit to a one compartment absorption and elimination model. Only one subject had detectable free curcumin at any of the 14 time points assayed. However, curcumin glucuronides and sulfates were detected in all subjects. Based on the pharmacokinetic model, the area under the curve for the 10 and 12 g doses was estimated as 35.33 and 26.57 μg/mL x hour, respectively, whereas C_{max} was 2.30 and 1.73 μg/mL. The T_{max} and $t_{1/2}$ were estimated to be 3.29 and 6.77 hours. The ratio of glucuronide to sulfate was 1.92:1. The results of this study showed that at high doses (10-12 g) curcumin is absorbed following oral administration in humans and can be detected as both glucuronide and sulfate conjugates in plasma.

In another clinical trial, 25 subjects with conditions associated with a high risk of malignancy received increasing doses of curcumin (purity- 99.3%) for 3 months (Cheng et al., 2001). The starting dose was 500 mg/day, which was increased stepwise to 1000, 2000, 4000, 8000 and finally 12,000 mg/day. In patients taking 500 to 2000 mg doses, serum curcumin was barely detectable, while in patients taking 4000 to 8000 mg curcumin, serum concentrations of curcumin peaked at 1 to 2 hours and gradually declined within 12 hours, although a half-life was not determined. The average peak serum concentrations after taking 4000 mg, 6000 mg and 8000 mg of curcumin were 0.51, 0.63 and 1.77 μM, respectively. No curcumin was detected in urine. Similar results were obtained in two patients who took curcumin for more than 1 month, indicating that repeated administration did not alter the pharmacokinetic profile of this substance and that no accumulation had occurred. The low blood levels of curcumin seen in these studies and the absence of curcumin seen with lower doses are consistent with extensive metabolism of curcumin in the intestinal wall and/or poor absorption. The possible differences noted in bioavailability may also be related to synthetic (Cheng et al., 2001) versus natural curcumin containing other curcuminoids (Sharma et al., 2004).

Kanai et al. (2012) evaluated the pharmacokinetics of a newly developed nanoparticle curcumin with increased water solubility. In this study, six healthy human volunteers (five men

and one woman; mean age, 44 years; range, 38–51 years; mean body mass index, 24.4; range, 20.2–27.8) received a single oral dose of 150 mg curcumin. After an interval of 2 weeks, the same subjects then received curcumin at a single oral dose of 210 mg. Plasma curcumin levels were measured at 0, 1, 2, 4, 6, and 24 h after curcumin ingestion. C_{max} for curcumin at 150 and 210 mg was 189 and 275 ng/ml, respectively, and the area under the curve (AUC) for 24 hour was estimated to be 2,649 and 3,649 ng/ml × hour, respectively. The $t_{1/2}$ was estimated to be 9.7 hour for 150 mg and 13 hour for 210 mg. One subject reported grade 1 diarrhea after intake of 150 mg curcumin but this did not occur after the second 210 mg dose. No other adverse effects were observed in this study. The results of this study indicate that plasma curcumin levels can be safely increased in a dose-dependent manner at a dose up to 210 mg without saturating the absorption system.

The available evidence from clinical trials performed in healthy as well as cancer subjects has demonstrated low systemic bioavailability of curcumin following oral administration, even at doses of up to 12 g/day (Cheng et al., 2001; Dhillon et al., 2008). Some investigators have attempted to increase the bioavailability of curcumin by dosing with glucuronidation inhibitors such as piperine. These investigations revealed increased bioavailability of curcumin in humans (Shobha et al., 1998). In a recent pharmacokinetic study in humans, Gota et al. (2010) reported relatively higher bioavailability of standardized novel solid lipid curcumin particle (SLCP) preparation compared to generic curcumin extract. In this single-dose, crossover, double-blind, comparative pharmacokinetic study both SLCP and generic curcumin extract (95% curcuminoids) were administered to six male healthy volunteers (18 - 40 years) at a dose levels of 650 mg/person and the C_{max} for these two curcumin preparations was found to be 22 and <1 ng/ml, respectively. These observations from the human study suggest the potential for sustained release for lipid-based formulations such as SLCP.

2.2. Acute and Short-term Studies

Oral (gavage) administration of single doses of 1380 to 3500 mg/kg bw of commercially available curcumin to rats (sex, strain not mentioned) produced no adverse effects aside from discoloration of the feces. The results of this study suggest that LD_{50} of curcumin is greater than 3500 mg/kg body weight (Anonymous, 1996). In two other studies, oral administration of a 2000 mg curcumin/kg (Shoba et al., 1998) to male and female Wistar rats or up to 5000 mg/kg (Wahlstrom and Blennow, 1978) to Sprague Dawley rats did not reveal any discernible adverse effects. Similarly, the acute oral LD_{50} of curcumin oil was reported to be greater than 5000 mg/kg in rats (sex, strain not mentioned) while the acute oral LD_{50} of curcumin in mice (sex, strain not mentioned) was reported as greater than 2000 mg/kg (Srimal and Dhawan, 1973; Opdyke and Letizia, 1983). The acute LD_{50} of a turmeric extract containing ~79% curcumin was reported to be greater than 10 g/kg in both rats and mice (Lilja et al., 1983). The oral LD_{50} of solid lipid curcumin particle (SLCP) preparation in rats as well as in mice was found to be greater than 2000 mg/kg (Dadhaniya et al., 2011).

2.3. Subchronic Studies

Based on the results of an unpublished subchronic study by Central Food Technological Research Institute (Mysore, India) and National Institute of Nutrition (Hyderabad, India) (CFTRI, 1978), dietary administration of curcumin at a dose level of 0.1, 0.5, 1 and 2% in feed to 10 male rats for 8 weeks did not reveal any adverse effects. In another study, no adverse effects as evaluated by growth, behavior, biochemical or histological parameters were seen in rats and

monkeys fed 1 .8 g/kg body weight/day and 0.8 g/kg body weight/day, respectively, for 90 days (Majeed et al., 1995). In yet another study, feeding of whole spice turmeric or curcumin to rats at doses equal to or much higher (1.25- to 125-fold) than those normally consumed by humans for 8 weeks did not reveal any adverse effects on growth, feeding efficiency, or hematologic parameters. At the highest dosage (10% curcumin equivalent dose ~ 5000 mg/kg body weight) decreased food consumption and subsequent lower feeding efficiency were noted. However, this was thought to be associated with or due to the effect of curcumin on food palatability (Sambaiah et al., 1982). Additional details of these studies were not available.

Curcumin has been under development as an anticancer agent by the Chemoprevention Branch of the National Cancer Institute since 1988. Under this program, in two separate subchronic studies, the safety of curcumin was evaluated in rats and dogs. In the rat study, oral (gavage) administration of curcumin at dose levels of 1140, 1515, 1995, 2630, and 3500 mg/kg body weight/day for 90 days revealed colored feces and yellow fur in rats. In some males, decreased reticuloycyte counts and increased mean corpuscular hemoglobin were noted. These changes were not considered to be biologically significant. In male rats at dose levels of 1515 mg/day and higher, and in female rats at the highest dose, small decreases in body weights were observed. The no-observed adverse effect level (NOAEL) was considered to be >3500 mg/kg body weight/day (Anonymous, 1996). In the dog study, administration of 250, 500, and 1000 mg/kg body weight/day curcumin in a gelatin capsule formulation for 90 days to male and female dogs resulted in significant elevations in mean corpuscular hemoglobin concentration in the mid- and high- dose females. As no overt anemia was detected, these elevations were not considered to be biologically relevant. The NOAEL was thus considered to be >1000 mg/kg body weight/day in dogs (Anonymous, 1996).

The National Toxicology Program (NTP) conducted two well-designed subchronic toxicity studies in rodents using a turmeric oleoresin with high curcumin content (79 to 85%). F344 rats (10/group/sex) and B6C3F1 mice (10/group/sex) were maintained on diets containing a turmeric oleoresin (approximately 79% curcumin) at concentrations of 0, 0.1, 0.5, 1.0, 2.5, or 5.0% for 13 weeks. In rats, these levels were estimated to deliver turmeric oleoresin of 0, 50, 250, 480, 1300, 2600 mg/kg body weight/day in males and 0, 60, 300, 550, 1450 and 2800 mg/kg body weight/day in females. In mice, the resulting intake of the oleoresin was estimated as 0, 150, 750, 1700, 3850, 7700 mg/kg/day in males and 0, 200, 1000, 1800, 4700 and 9300 mg/kg/day in females.

In the rat study, no significant treatment-related differences were noted in body-weight gain, mortality, or histopathology. A dose-related increase in liver weight was observed in both sexes. In female rats, a treatment-related decrease in heart and lung weights was noted. Hematological examination revealed a dose-related increase in polymorphonuclear lymphocytes at the top two dose levels in males, while in females there was a small increase only at the highest dose level. In females, hematological parameters did not reveal any dose-related changes except in erythrocyte counts which tended to be lower in a treatment-related, but not a consistent dose-related manner. In male rats, clinical chemistry analyses revealed a number of changes at the mid- to high-dose levels; serum glutamic pyruvic transaminase (SGPT), ornithine carbamoyltransferase (OCT), 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 controls. At the highest-dose level, decreased serum glutamic oxaloacetic transaminase (SGOT) and lactate dehydrogenase (LDH) levels were noted. In female rats, decreases were

noted in LDH, creatinine, total bilirubin, pH, bicarbonate, and total $C0_2$, while phosphorus was increased at the two higher-dose levels. In male rats, urinalysis revealed a treatment-related increase in casts and an increase in red blood cells at the top two dose levels. In females, urine 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 highest-dose level. The no-effect level with respect to gross and microscopic pathological changes was 5% of the diet, equal to a time-weighted average of 2760 and 2587 mg/kg body weight/day in females and males, respectively (Lilja et al., 1983).

In the mouse study, no significant treatment-related differences were noted in body weight gain, mortality, or histopathology. However, a dose-related increase in liver weight occurred in both sexes. Additionally, decreases in lung weights were noted that achieved statistical significance in males at the two highest dose groups only, in thymus weight, significant only at the 2.5% level, and in kidney weight, significant in females of the highest dose group only. No dose-related changes were noted in hematological parameters and the values were within normal ranges. Clinical chemistry analyses revealed dose-related increases in cholinesterase and phosphorus. The increases were significant at the 1% and higher dose levels in males and at the top two dose levels (cholinesterase) or at the highest dose group only (phosphorus) in females. In females, a dose-related decrease in creatinine levels was noted in all but the lowest dose level. In males, a decrease in creatinine was noted at the top three dose levels. The no-effect level with respect to gross and microscopic pathological changes was 5% of the diet, equal to a time-weighted average of 9280 and 7700 mg/kg body weight/day in females and males, respectively (Lilja et al., 1983).

In a recent study, Dadhaniya et al. (2011) investigated the effects of a solid lipid curcumin particle (SLCP) preparation in rats following subchronic administration. In this study, Wistar rats (10/sex/group) were administered via oral gavage 0 (control), 180, 360, and 720 mg/kg bw/day of SLCP preparation for 90 days. Administration of the curcumin preparation did not result in any toxicologically significant treatment-related changes in clinical (including behavioral) observations, ophthalmic examinations, body weights, body weight gains, feed consumption, and organ weights. No adverse effects of the curcumin preparation were noted on the hematology, serum chemistry parameters, and urinalysis. Terminal necropsy did not reveal any treatment-related gross or histopathology findings. Based on the results of this study, the NOAEL for curcumin preparation was determined as 720 mg/kg bw/day, the highest dose tested.

2.4. Chronic Toxicity and Carcinogenicity Studies

The National Toxicology Program (NTP, 1993) also conducted a 2-year chronic toxicity and carcinogenicity study with turmeric oleoresin (79% curcumin) in rodents. F344/N rats (60/sex/group) were fed diets containing 0, 2000, 10,000, or 50,000 ppm turmeric oleoresin for 104 weeks (males) or 103 weeks (females). Diets were estimated to deliver average doses of 0, 80, 460, or 2000 mg/kg body weight/day for male rats and 0, 90, 440, or 2400 mg/kg body weight/day for female rats. At the end of 15 months, nine or ten animals from each group were euthanized for interim evaluation. Feeding of turmeric oleoresin did not affect survival rates of male and female rats. Compared to control, final mean body weights were not affected at the 2000 and 10,000 ppm levels. In the high-dose group, the mean body weights were slightly lower (up to 10%) in both sexes throughout much of the study without any changes in feed consumption. At the 15-month interim evaluation, significant increases in the absolute and relative liver weights were seen in female rats at the 10,000 and 50,000 ppm dose levels. No

clinical findings related to toxicity were noted. Hematological evaluation at the interim evaluation revealed significantly reduced hematocrit values, hemoglobin concentrations, and erythrocyte counts in high dose males and females. In the high dose group, platelet counts were significantly increased in both males and females while a significant increase in reticulocyte counts was noted in males only. Clinical chemistry parameters did not reveal any biologically significant changes.

In the gastrointestinal tracts of high dose rats, non-neoplastic lesions were observed. In males, an increased incidence of ulcers, hyperplasia, and hyperkeratosis of the forestomach was noted, while ulcers, chronic active inflammation, and hyperplasia of the cecum were noted in both sexes. Similar lesions were seen in the colon of males. Compared to controls, increased incidences of sinus ectasia of the mesenteric lymph node were noted at the 10,000 ppm dose level in males and at the 50,000 ppm dose level in both sexes. These lesions were considered likely to be regenerative and not neoplastic in nature.

In male F344/N rats, no evidence of carcinogenic activity was noted following exposure to turmeric oleoresin. In females, an increased incidence of clitoral gland adenomas was seen in all turmeric oleoresin exposed groups. Clitoral gland carcinomas were observed in one control animal and in four rats at the 2000 ppm dose level. Notably, the marginal increase of clitoral gland adenoma was neither dose-related nor associated with a corresponding increase in hyperplasia. No animals at the 10,000 or 50,000 dose level were affected. The combined incidences of clitoral gland adenoma or carcinoma in all exposed groups of female rats were similar and did not increase with exposure level. The incidence of clitoral gland hyperplasia was similar among exposed and control groups of female rats.

In the 2-year chronic toxicity and carcinogenicity study in mice, male and female B6C3F1 mice (60/sex/group) were fed diets containing 0, 2000, 10,000, or 50,000 ppm turmeric oleoresin for 104 weeks (males) or 103 weeks (females). The estimated daily doses of male and female mice were determined to be 0, 220, 520, or 6000 mg/kg and 0, 320, 1620, or 8400 mg/kg, respectively. Nine or ten animals from each group were euthanized after 15 months of treatment for interim evaluation. Feeding of turmeric oleoresin did not affect survival rates of male and female mice. Compared to the control group, the mean body weights of females at the high dose level was slightly lower (up to 12%) starting from approximately week 25. At study termination, in male rats fed 50,000 ppm and females fed 10,000 and 50,000 ppm turmeric oleoresin, a significant decrease in mean body weights were noted. Throughout the study, feed consumption in all exposed groups was similar to controls. The 15 month interim evaluation revealed a significant increase in the absolute and relative liver weights in both males at females in the 10,000 and 50,000 ppm dose groups. Clinical observations did not reveal any toxicologically significant findings. Hematological parameters did not reveal any biologically significant differences. Clinical chemistry parameters revealed significant higher levels of alkaline phosphatase values in males and females at the 10,000 and 50,000 ppm levels (NTP, 1993).

A significant increase in the incidence of hepatocellular adenoma was noted in males and females at the 10,000 ppm dose level, but not at the 2000 or 50,000 ppm levels. Compared to control rats, the number of male and female mice in the 10,000 and 50,000 ppm groups with multiple hepatocellular neoplasms was significantly greater. The incidences of hepatocellular carcinoma were similar among control and exposed groups. In contrast to rats, there were no turmeric oleoresin-related non-neoplastic lesions of the gastrointestinal tract in mice. Three males each in the 2000 and 10,000 ppm dose groups had carcinomas of the small intestine

however no such pathology of the small intestine was noted at the 50,000 ppm dose level. At the high level, a significantly increased incidence of thyroid gland follicular cell hyperplasia was noted in female mice (NTP, 1993).

In summary gastrointestinal irritation (ulcers, hyperplasia and inflammation) was common in rats in the high-dose group, but was not observed in mice. The no observed effect level (NOEL) for gastrointestinal effects in rats was 10,000 mg/kg in the feed, equal to 440 mg/kg bw/day. After 15 months of treatment, absolute and relative liver weights were increased in both male and female mice in the mid- and high-dose groups relative to control. The NOEL for liver enlargement was 2000 mg/kg in the diet, equal to 220 mg/kg body weight/day (JECFA, 1994). The ingestion of turmeric oleoresin was also associated with thyroid gland follicular cell hyperplasia in females (NTP, 1993). The NTP report concluded that under conditions of the two year feeding study, there was no evidence of carcinogenic activity of turmeric oleoresin in male F344 rats administered 2000, 10,000, or 50,000 ppm. In female rats, there was equivocal evidence of carcinogenic activity based on increased incidences of clitoral gland adenomas in the groups exposed to turmeric oleoresin. In B6C3F1 mice, there was equivocal evidence of carcinogenic activity in males based on a marginally increased incidence of hepatocellular adenoma at the 10,000 ppm level and the occurrence of small intestine carcinomas at the 2000 and 10,000 ppm groups. Similarly, in female mice, there was equivocal evidence of carcinogenic activity based on the increased incidence of hepatocellular adenoma at the 10,000 ppm dose level (NTP, 1993). The JECFA reviewed the results of the NTP studies and concluded that 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 reported, the effects were not dose-related, and that curcumin was not a carcinogen (WHO, 1996).

2.5. Genotoxicity Studies

Curcumin or turmeric oleoresin was not mutagenic in most systems in which it was tested. Several investigators have evaluated the potential genotoxic effects of curcumin in both *in vitro* and *in vivo* mutagenicity assays. Curcumin was not mutagenic in the Salmonella Ames assay or the mouse dominant lethal assay with or without metabolic activation (Ishidate et al., 1981, 1984; Jensen 1982; Mortelmans et al., 1986; Vijayalaxmi, 1980; Shah and Netrawali, 1988; Giri, 1991), and was negative in the yeast gene conversion test (Sankarnarayanan and Murthy, 1979). Curcumin was also negative in Chinese Hamster Ovary (CHO) cells (Au and Hsu, 1979), and did not induce micronuclei or dominant lethal mutations (Vijayalaxmi, 1980).

In some *in vitro* and *in vivo* assays of clastogenicity, equivocal results of curcumin have been reported (Ishidate et al., 1988; Giri, 1990). In several *in vivo* and *in vitro* assays with plant and animal cells, curcumin was found to induce clastogenicity (Abraham et al., 1976; Goodpasture and Arrighi, 1976; Ishidate et al., 1984). In cultured hamster fibroblasts, curcumin was found to induce chromosomal aberrations (Kawachi et al., 1980; Ishidate et al., 1984). In the *Bacillus subtilis* Rec assay, Kawachi et al. (1980) reported growth inhibition due to DNA damage. In a single cell electrophoresis method (Comet Assay), Blasiak et al. (1999) reported that exposure of gastric mucosa cells and human peripheral blood lymphocytes to curcumin at concentrations of 15, 25, and 50μM resulted in DNA damage. However, the damaged cells were able to recover within a period of 2 hours.

In two recent studies, Birrell et al. (2010) and Fowler et al. (2011) addressed the question of false positive results of chemicals, including curcumin. Birrell et al. (2010) tested chemicals including curcumin in the GreenScreen HC assay, while Flower et al. (2011) compared several rodent cell lines (V79, CHL, CHO) with p53-competent human peripheral blood lymphocytes (HuLy), TK6 human lymphoblastoid cells, and the human liver cell line, HepG2. Based on the results of these investigations, Birrell et al. (2010) suggested that curcumin is an antioxidant that can act as a pro-oxidant in the hyperoxic conditions of cell culture.

In an earlier *in vivo* study, feeding a diet containing curcumin (0.015%) for 12 weeks to mice did not result in the induction of chromosomal aberrations, micronuclei, or dominant lethal mutations (Vijayalaxmi, 1980). Giri et al. (1990) investigated changes in bone marrow cell sister chromatid exchanges (SCEs) and chromosomal aberrations following acute and chronic dietary exposure of curcumin and tartrazine to 10-to 12-week- old Swiss albino male mice and rats. Curcumin was weakly clastogenic *in vivo* in mice. No significant increase in chromosomal aberrations was noted in the curcumin-treated animals, whereas tartrazine showed a significant increase in chromosomal aberrations in some of the higher concentrations. Giri et al. (1990) also reported that curcumin (unknown purity) induced SCE at a low frequency above 25 mg/kg body weight in mice following intraperitoneal administration, while in rats fed curcumin of unknown purity, there was equivocal evidence for the induction of chromosomal aberrations. In another study (Anonymous, 1996), single intraperitoneal injections of 25 to 200 mg/kg body weight of curcumin to Swiss mice increased significantly SCEs in bone marrow cells. Although significant increases in SCEs were noted, no dose achieved increases that were twice the background rate. In contrast to some of the results described above, several studies have indicated that curcumin possesses anti-mutagenic activity (JECFA, 1994).

In summary, several studies investigated the genotoxic effects of curcumin and turmeric. In the Salmonella Ames assay or the mouse dominant lethal assay with or without metabolic activation, curcumin was not mutagenic. In clastogenicity assays, equivocal results of curcumin have been reported. Overall there is inadequate evidence for the genotoxicity of curcumin. The SCE data in particular was considered to be of little relevance in the evaluation (JECFA, 1994), while other studies could not be reliably interpreted because of impurities in the curcumin preparations used.

2.6. Reproductive and Developmental Toxicity

Based on unpublished data (cited in Govindarajan, 1980), administration of curcumin at dose levels of 600 and 1600 mg/kg body weight, respectively, to rats and rabbits on days 6 through 15 of gestation did not reveal any adverse effects on implantation, resorption, embryo survival, or skeletal or visceral abnormalities. Similarly, feeding of male and female rats diets containing 0.5% (~250 mg/kg) or 0.015% (~75 mg/kg) curcuminoids for 12 weeks prior to mating did not reveal any adverse effects on pregnancy rate, mean number of live and dead embryos, or total implants in rats (Govindarajan, 1980). Vijayalaxmi (1980) also reported that dietary administration of 0.015% curcumin to rats for 12 weeks had no effects on pregnancy rate, embryo viability, total implantations, or mutagenic index.

In a two-generation study (Ganiger et al., 2007; also cited in JECFA, 2004), groups of Wistar rats (30/sex/group) were fed diets containing curcumin at a concentration of 0, 1500, 3000 or 10000 mg/kg of diet starting from 10 weeks before the mating period and continuing throughout mating. The study was designed and conducted in accordance with OECD guidelines.

The concentrations used corresponded to doses of 0, 130 to 140, 250 to 290 or 850 to 960 mg/kg body weight/day in males, and 0, 160, 310 to 320 or 1000 to 1100 mg/kg body weight/day in females. Females were fed the curcumin containing diet throughout the pregnancy and weaning of the offspring. The total periods of treatment were 21 weeks for the parental F_0 generation and 24 weeks for the F_1 generation. The litter sizes of the F_1 offspring were standardized to a maximum of eight on postnatal day 4. After weaning, 30 male and 30 females of the F_1 generation were selected for breeding. Various reproductive measures (i.e., fertility indices, litter sizes, postimplantation loss, and survival indices) were calculated. The data of body weight, body weight gains, food consumption, mean number of implantations and mean litter size were compared by Bartlett's test for homogeneity of intra group variances. When the variances were heterogeneous, the data was transformed using appropriate transformation. Pups were weighed on postnatal days 1, 4, 7, 14 and 21. All parents, F_1 weanlings not selected for mating, and all F_2 weanlings were subjected to complete necropsy at termination. The primary and secondary sex organs, liver, kidney, pituitary and adrenal glands were removed at necropsy from all parental animals, male and female. The tissues were processed and stained with hematoxylin and eosin and subjected to histopathological examination (Ganiger et al., 2007).

During the course of study, there were no treatment related clinical signs of toxicity, ophthalmological changes or mortality. During the premating period, there were no treatment related effects in group mean body weights and net body weight gains or food intake between treated and control animals of either generation. Some isolated, statistically significant changes noted were not considered as treatment-related. In either generation, there were no differences in gestational or postpartum body weights or food consumption. In the F0 generation, maternal body weight gain during the lactation period was significantly higher in the 3000 and 10000 ppm dose groups when compared with controls. Among the offspring of both generations, there were no differences in mean litter size and mean viable litter size at birth, live birth index, and survival indices on days 4, 7, 14 and 21. In F1 generation, there was a small but significant increase in pup weight observed with the 3000 ppm dose on postpartum day 1 and at the 10,000 ppm dose on postpartum days 1, 4 and 7. In the F2 generation, reduction in pup weight was observed at the 3000 ppm dose on postpartum day 7 and with the 10,000 ppm dose on postpartum days 7, 14 and 21. The live birth index was significantly higher in the 3000 and 10,000 ppm dose levels in the F1 generation and lower in the 3000 ppm dose level in the F2 generation, but these differences were small and not of any biological significance. No statistically significant differences were observed between control and treated parental animals of both generations for male and female fertility indices, fecundity index, parturition percentage, post implantation loss and percentage of live pups born. No treatment-related gross or histopathological changes were observed in any of the animals. Based on the results of this study, the investigators concluded that there were no adverse toxicological effects of curcumin on the reproductive parameters and the NOAEL for reproductive toxicity was determined as 10,000 ppm in diet (equivalent to equivalent to 847 and 959 mg/kg body weight per day for male rats and 1043 and 1076 for females) (Ganiger et al., 2007).

Prior to the publication of Ganiger et al. (2007) study, JECFA (2004) at its 61st meeting reviewed this study submitted to World Health Organization by Spices Research Foundation, Cochin, India (Ganiger, 2002). The JECFA group considered that the small body weight reduction in the F_2 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 as adverse effects. In its evaluation JECFA (2004) stated, "There was a dose-related decrease in

body-weight gain in the dams of the parental generation during days 10–15 of gestation, which was statistically significantly different from that of controls (body-weight gains, >80% that of controls) at the intermediate and highest doses. 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 F_2 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; and therefore this dose, equal to 250–320 mg/kg bw per day for the F_1 generation, was the NOEL (Ganiger, 2002)." The data (small pre-weaning decrease in body weight gain in the F2 pups at the highest dose) that formed the basis of JECFA (2004) determination of NOEL (no-observed effect level) and reported in Ganiger et al. (2007) is presented in Table 7 to draw some conclusions.

Table 7. Body weight (g) of F1 and F2 pups during lactation period (Ganiger et al., 2007)

	F0 → F1 generation				F1 → F2 generation			
	0	**1500**	**3000**	**10000**	**0**	**1500**	**3000**	**10000**
Day 1	61.±0.4	6.4±0.5	6.6±0.5*	6.7±0.4*@	6.9±0.4	6.6±0.7	6.5±0.6*	6.6±0.6
Day 4	9.1±0.9	9.3±0.9	9.7±1.2	10.0±1.0*	10.4±0.8	9.9±1.2	9.7±1.1	9.9±1.1
Day 7	14.1±1.2	14.2±1.1	14.9±1.6	15.1±1.1*	16.6±1.1	15.3±1.6	15.0±1.6*	15.0±1.2*
Day 14	28.5±2.3	27.8±1.5	28.5±2.3	28.7±2.0	29.5±1.9	29.4±2.0	28.8±2.7	28.0±2.0*
Day 21	41.1±4.2	41.5±2.9	43.9±3.2	44.2±2.8	45.4±3.2	44.3±3.3	44.1±4.6	42.5±3.1*

Doses (ppm): 0, 1500, 3000, 10000; * = Significant at $P \leq 0.05$; @ = Significant dose correlation.
Adapted from Ganiger et al., 2007

From Table 7, it can be noted that the preweaning decrease in body weight gain of F2 generation was marginal. A look at the data from control groups (0 ppm) of both F1 and F2 pups also indicate a notable difference. Compared to the F1 control group, there appears to be a significant increase in the F2 control group. It is not clear why an increase in control group between F1 and F2 generation was noted. It is anticipated that there should be no difference between F1 and F2 control group pups. The contribution of higher body weights of F2 generation control group compared to F1 generation to the statistically significant decrease in pup weights in F2 generation is not clear. Additionally, JECFA (2004) also recognized that the effect was small and the observed changes were reported to be within the range of the data for historical controls. Thirdly, curcumin administration at levels of up to 10000 ppm did not affect any of the reproductive parameters such as male or female mating performance, fertility, postimplantation loss, parturition, mean litter size and mean viable litter size at birth, survival indices, or histopathological changes in either generation. In light of this the NOEL of 250-320 mg/kg body weight determined by JECFA appears to be low. A more appropriate and reasonable NOAEL, also determined by Ganiger et al. (2007), should be 847 - 1076 mg/kg body weight/day. It is possible that the NOAEL may be even higher.

2.7. Sensitization and Allergenicity

Liddle et al. (2006) reported two cases of contact urticaria from curcumin. A 44-year-old woman was evaluated following a recent pruritic eruption on the face and exposed areas of the

arms. Results of open testing performed with curcumin were negative. Subsequent, skin prick testing with curcumin resulted in a large pruritic 2.5 cm red patch 30 to 40 minutes after application. The patient was diagnosed with nonimmunologic contact urticaria from curcumin. In another skin prick test with curcumin, a 20 year old woman developed a large area (> 2 cm) of redness within minutes of exposure followed by intense pruritus that progressed up her arm and became generalized. The investigators suggested that these two cases of contact urticaria from curcumin were apparently produced by two completely different mechanisms, one immunologic and the other nonimmunologic.

Goh and Ng (1987) reported a case of a 64-year old male Indian spice worker with allergic contact dermatitis to turmeric (*C. longa*). The worker was reportedly exposed to 7 different spices and worked in a dusty place laden with spice powders. No quantitative estimate of exposure was available. The authors concluded that while turmeric is a weak skin sensitizer, allergic contact dermatitis to turmeric is uncommon. Other case reports of allergic contact dermatitis due to curcumin have also been reported in the literature. Hata et al. (1997) described a woman who developed erythema, papules and vesicles due to curcumin exposure. Kiec-Swierczynska (1998) reported a case of occupational allergic contact dermatitis due to curcumin food color in a pasta factory worker. Futrell and Rietschel (1993) tested a series of fifty-five patients with suspected contact dermatitis for sensitivity to a group of spices, including turmeric at concentrations of 10 and 25% in petrolatum. Two subjects showed positive reaction to turmeric at a concentration of 25%. No reactions to turmeric were noted at the lower (10%) concentration. The authors noted that it was unclear whether this was indicative of a threshold for detecting true allergy or a marginal irritant reaction. In a review of information on natural color additives and foods, Lucas et al. (2001) noted some reactions in double-blind, placebo controlled food challenges involving mixtures of natural colorings. However, it was not possible to determine which of the food colorants may have triggered the adverse reactions. They concluded that no convincing evidence exists of allergic reactions to turmeric or curcumin ingestion from food colors (Lucas et al., 2001).

2.8. Human Studies

Several clinical trials have been conducted with oral administered curcumin or turmeric extract to humans and reported in the literature. The objectives of the majority of these studies were to examine the antioxidant, anti-inflammatory, anti-cancer, or anti-atherogenic effects of curcumin or turmeric extract. Although the primary end point of these investigations was to study their efficacy, clinical observations also included any reported adverse effects. These studies provide an indirect opportunity to access the safety and 'tolerability' of curcumin and turmeric extract in a diverse population. Three different Phase I clinical trials performed to determine safety indicate that curcumin given at doses as high as 12 g/day orally for 3 months is safe (Cheng et al., 2001; Goel et al., 2008; Aggarwal and Sung, 2008). No dose-limiting toxicity was reported. A summary of safety-related clinical trials including their design, doses used and reported adverse effects observed in these investigations with oral curcumin or turmeric extract treatment is given in Table 8.

Table 8. Summary of safety-related clinical trials of curcumin and turmeric extract

Reference	Number of subjects	Dose, Duration	Adverse Effects Reported/ Results
Studies with curcumin			
Deodhar et al., 1980	18 (16 F; 2M); ages 22-48 yrs; suffering from 'definite' rheumatoid arthritis	1.2 g/day in divided three doses; Double-blind cross over study	No adverse effects reported by the subjects. No significant changes in blood pressure, pulse, hemoglobin, hepatic an renal function
Lal et al., 1999	53; ages 19-70; suffering from chronic anterior uveitis (CAU)	375 mg three times daily for 12 weeks	32 of the 53 completed. None of the patients reported any adverse effects.
Lal et al., 2000	8 (5M; 3F); ages 6-54; suffering from idiopathic inflammatory orbital psuedotumors	375 mg three times daily for 12 weeks	5 of the 8 completed study. No side effects were noted in any patients.
Cheng et al., 2001	25 patients with high risk conditions such as recently resected urinary bladder cancer, arsenic brown's disease of skin, CIN, oral leukopekia or intestinal metaplasia	Dose escalation from 0.5 to 1, 2, 4, 8 and 12 g/day for 3 months	No toxicity at doses up to 8 g/day. Above 8 g the bulky volume of the test substance was unacceptable to the subjects. Peak serum levels of curcumin noted at 1-2 hrs and gradually declined by 12 hrs.
Sharma et al., 2004	15 adult colorectal cancer patients	0.45, 0.9, 1.8, 3.6 g/day for up to 4 months (curcumin C3 Complex)	Well tolerated. One subject consuming 0.45 g/day had NCI grade 1 diarrhea while another subject (3.6 g/day) experienced NCI grade 2 diarrhea. A third patient (0.9 g) experienced NCI grade 1 nausea which resolved spontaneously. At the highest dose detectable levels of curcumin and metabolites in plasma and urine, and inhibition of PGE2 production in blood leukocytes were noted
Lao et al., 2006	24 (3/group); healthy adult	0.5, 1, 2, 4, 6, 8, 10 or 12 g; single administration; dose escalation study	7 of 24 subjects (30%) experienced only minimal toxicity that did not appear to be dose-related. No other effects were noted
Ringman et al., 2012	36 (12/group); mild-to-moderate probable Alzheimer's disease	0, 2, and 4 g/day (Curcumin C3 Complex®) for 24-week with an open-label extension to 48 weeks	Two subjects receiving 2 g and one receiving 4 g withdrew due to GI side effects. No serious adverse events. Overall, no advesre effects between control and treatment groups.

Reference	Number of subjects	Dose, Duration	Adverse Effects Reported/ Results
Irving et al., 2012	26; positive fecal occult blood as part of colorectal screening program, awaiting diagnostic or surveillance endoscopy or diagnosis of colorectal cancer	5 x 470 mg capsules (total 2.35 g curcuminoids)/day for 14 days	Curcuminoids were detectable in nine of 24 plasma samples, 24 of 24 urine samples, and in the colonic mucosa of all 23 biopsied participants. Mean tissue levels were 48.4 mg/g (127.8 nmol/g) of parent curcuminoids. Curcumin glucuronide, was detectable in 29 of 35 biopsies. High levels of topical curcumin persisted in the mucosa for up to 40 hours post-administration.
Turmeric extract			
Kositchaiwat et al., 1989	60 (M; F); gastric ulcer; 30/group; ages 18-80 years	Turmeric; 1000 mg/day (250 mg capsule four times daily) for 12 weeks	No adverse effects noted. In two patients increased alkaline phosphatase levels were noted but could not be proven to be treatment-related.
Sharma et al., 2001b	15 with advanced colorectal cancer	Between 0.44 and 2.2 g/day for (36 and 180 mg curcumin/day) for up to 4 months	Well tolerated. One patient on 1.32 g/day experienced nausea during first month that resolved despite continuation of treatment. one patient on 0.88 and another on 2.2 g/day developed diarrhea at 4 and 1 month, respectively and withdrew
Prucksanand et al., 2001	45 (24M; 21F); ages 16-60 years	Turmeric; 3000 mg/day (600 mg five times daily) for 12 weeks	Study subjects were patients abdominal hunger pain, vomiting, hematomesis and melena. No serious adverse effects noted. Hematology and blood chemistry showed no significant changes.
Joshi et al., 2003	9 healthy volunteers; ages 20-33 years	Turmeric oil; 0.6 ml three times per day for 1 month and 1 ml in three divided doses for 2 months	One discontinued on day three due to allergic skin rash. Another discontinued on day 7 due to inter-current fever, unrelated to treatment
Bundy et al., 2004	207 subjects with IBS; 166 completed	72 or 122 mg of curcumin extract for 8 weeks; Partially blinded, randomized, two-dose, pilot study	No major side effects reported; minor side effects included flatulence, dry mouth; 9 subjects withdrew
M = male; F = female; IBS = Irritable bowl syndrome			

In a well designed dose escalation trial, Lao et al. (2006) attempted to determine curcumin's maximum tolerated dose (MTD) and safety. In this trial, a standardized powder extract of uniformly milled curcumin (Curcumin C3 Complex®, Sabinsa Corporation), was administered to 24 healthy volunteers as a single dose ranging from 500 to 12,000 mg. Remarkably, only minimal, non-dose-related toxicity was seen and this was observed in only seven subjects (30%). No curcumin was detected in the serum of subjects administered 500, 1000, 2000, 4000, 6000 or 8000 mg and only low levels of curcumin were detected in two subjects administered 10,000 or 12,000 mg.

Administration of single oral doses of up to 2 g curcumin to healthy human subjects did not reveal any evidence of adverse health effects (Anonymous, 1996; Shoba et al., 1998). In another study, Soni and Kuttan (1992) reported no adverse effects among ten subjects receiving 500 mg of curcumin (98% purity) daily for 7 days. In yet another study, no adverse effects were noted following multiple doses of 600 mg turmeric oil mixed with 3 g turmeric ethanol extract per day or 375 mg curcumin three times daily in three-month treatment duration clinical studies (Hastak et al., 1997; Lal et al., 1999). Similarly, no adverse effects were noted in subjects consuming 200 mg of a hydroalcoholic extract of *C. longa* (~20 mg curcumin/day) for 15 to 60 days (Ramirez-Bosca et al., 1997; 2000). In earlier studies by this same group of investigators, the same treatment regimen (~20 mg curcumin/day for 15-60 days) did not reveal any signs of apparent liver or kidney injury as evaluated by glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), gamma-glutamyltransferase (GGT), alkaline phosphatase, and total bilirubin. Similarly, blood coagulation parameters were unaffected (Ramirez-Bosca et al., 1995, 1997).

In a prospective Phase I study, Cheng et al. (2001) evaluated the safety and pharmacology of curcumin. In this study, 25 patients with a high risk of malignancy were given curcumin (diferuloylmethane; 99.3% purity) for 3 months. The starting dose was 0.5 g/day that was gradually increased to 1, 2, 4, 8, and finally 12 g/day. The patients received regular follow-up, including physical examinations, and analysis of hematology and blood chemistry parameters. At dose levels up to 8 g/day, no adverse effects were reported. The highest dose of 12 g/day could not be tolerated because of the bulky volume of the tablets (Cheng et al., 2001). In another trial, oral administration of curcumin at a dose level of 1.2 g/day to rheumatoid arthritis patients did not reveal any adverse effects. In this study, blood pressure, pulse, erythrocyte sedimentation rate, and renal and hepatic function were likewise unaffected (Deodhar et al., 1980). In a randomized multicenter, double-blind placebo-controlled trial in patients (n=89) with ulcerative colitis, 45 patients received curcumin 2 g/day) plus sulfasalazine or mesalamine, while the placebo group (n=44) received sulfasalazine or mesalamine for 180 days (Hanai et al., 2006). No adverse effects of curcumin were noted.

In a study by Srimal and Dhawan (1985), administration of curcumin at a dose level of 1500 mg/day for 4 to 6 weeks produced subjective improvement in osteoarthritis symptoms without any adverse effects. Lal et al. (1999; 2000) reported no adverse effects in clinical studies using a dose of 1125 mg/day. In another study, 44 post-surgical patients received either curcumin (1200 mg/day), phenylbutazone (300 mg/day) or placebo for 5 days. One patient in the curcumin group reported mild transient giddiness on postoperative day 3, while 1 patient in the placebo group complained of nausea on the first postoperative day. No changes in blood chemistry were noted (Satoskar et al., 1986). As cited in Chainani-wu (2003) of the 19 HIV patients given 2500 mg curcumin/day (duration not specified), two subjects reported some gastric irritation, including one with a past history of peptic ulcers. No adverse reactions or changes in blood chemistry parameters were noted. In a randomized, double-blind, crossover study, Rasyid and Lelo (1998) examined the effects of 20 mg curcumin on gall-bladder volume and function in healthy human volunteers. Curcumin was reported to stimulate contraction of the gall bladder. None of the subjects reported experiencing adverse effects.

Sharma et al. (2001b) conducted a dose-escalation study of a standardized *Curcuma* extract in patients with advanced colorectal cancer refractory to standard chemotherapy. In this study, 15 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. Three subjects each received doses of extract equivalent to 26, 72, 108, 144 or 180 mg curcumin/day. Three subjects reported gastrointestinal symptoms. During the first month of treatment, one patient receiving 108 mg curcumin/day experienced nausea, which resolved spontaneously without discontinuing the treatment. Two subjects receiving 72 and 180 mg curcumin/day, respectively, experienced diarrhea. The relation of these symptoms to the treatment was not clear as there were no parallel controls in the study and the clinical condition of the patients was poor.

In a double blind, placebo-controlled trial, Ringman et al. (2012) investigated tolerability of curcumin (Curcumin C3 Complex®) in subjects with Alzheimer's disease receiving curcumin for 24 weeks followed by an open-label extension to 48 weeks. In this study, 36 subjects (12/group) with mild-to-moderate Alzheimer's disease were randomized to receive placebo, 2 g/day, or 4 grams/day of oral curcumin for 24 weeks. For weeks 24 through 48, subjects that were receiving curcumin continued with the same dose, while subjects from the placebo group were randomized in a 1:1 ratio to 2 or 4 g curcumin/day. The primary outcome measures were incidence of adverse events, changes in clinical laboratory parameters and the Alzheimer's Disease Assessment Scale. One subject withdrew in the placebo (8%, worsened memory) and 5/24 subjects withdrew in the curcumin group (21%, 3 due to gastrointestinal symptoms). Curcumin was associated with lowered hematocrit and increased glucose levels that were clinically insignificant. There were no differences between treatment groups in clinical or biomarker efficacy measures. The levels of native curcumin measured in plasma were low (7.32 ng/mL). Among the curcumin-treated subjects, three withdrew in the 2 g/day curcumin group (two due to gastrointestinal side effects of black stools and diarrhea, one due to subsequent difficulty swallowing the pills), and two subjects withdrew in the 4 g/day curcumin group (one due to diarrhea and one due to difficulty swallowing the pills). Overall, adverse events occurred in 91.7% of placebo-treated subjects and 100% of curcumin treated subjects (not significant). Complete safety laboratory data at baseline and 24 weeks were available for 11 completers on placebo, 9 on 2 g/day and 9 on 4 g/day of curcumin. Controlling for baseline values, subjects on curcumin had statistically lower hematocrit (placebo = 42.9%, 2 gm = 41.8%, 4 gm = 42.3%; P = 0.014) and higher glucose levels (placebo = 85.9 mg/dL, 2 gm = 91.6 mg/dL, 4 gm = 90.5 mg/dL, P = 0.043) while being treated. A subset of subjects underwent bleeding time assessments and no significant effect of curcumin was observed. The reason for the observed increased plasma glucose levels in persons treated with curcumin is unclear. However, these changes in hematocrit and blood glucose were relatively minor and values did not exceed the range of normal. The results of this study indicate that curcumin is well tolerated.

Irving et al. (2012) investigated the acceptability and compliance of daily curcumin in patients undergoing colorectal endoscopy or surgical resection. For this study 28 patients (13 male; 15 female; age- 35-85 years) were recruited. The subjects received 5 x 470 mg capsules (total 2.35 g curcuminoids)/day for 14 days. In this study, 24 of the 26 patients commencing curcumin completed the course. Six patients reported mild gastrointestinal adverse events. Sixteen participants (67%) stated that they would take curcumin long-term should it be of proven benefit. The investigator concluded that findings from this study provide further support for the safety of curcumin and its potential usefulness in long-term colorectal cancer prevention strategies.

In addition to these safety-related clinical trials, several other human studies with curcumin have appeared in the published literature. Recently, in an extensive review article, Goel et al. (2008) described ~40 completed and ~30 ongoing Phase I and II efficacy-related trials. These and some other trials reported in the literature are summarized in Table 9 and Table 10, respectively.

Table 9. Summary of human clinical trials of curcumin and turmeric extract

Disease	Dose; Frequency	Patient	Results; Reference
Rheumatoid arthritis	1200 mg/day x 14 days	18	Improved symptoms; Deodhar et al., 1980
Postoperative inflammation	400 mg; 3x/day x 5 days	46	Decrease in inflammation; Satoskar et al., 1986
External cancerous lesions	1% ointment x several months	62	Reduction in lesion size and pain in 10% patients; Kuttan et al., 1987
Cardiovascular	500 mg/day x 7 days	10	Decreased serum lipid peroxidase (33%) and total cholesterol (12%, increased HDL cholesterol (29%); Soni and Kuttan, 1992
Atherosclerosis	10 mg; 2x/day x 28 days	12	Lowered LDL and ApoB, increased HDL and ApoA; Ramirez-Bosca et al., 2000a
HIV	625 mg; 4x/day x 56 days	40	Well tolerated; James, 1996
Gall bladder function	20 mg, single dose (2 h)	12	Decreased gall bladder volume by 29%; Rasyid and Lelo, 1998
Gall bladder function	20–80 mg, single dose (2 h)	12	Decreased gall bladder volume by 72%; Rasyid et al., 2002
Chronic anterior uveitis	375 mg; 3x/day x 84 days	32	Eighty-six percent decrease in chronic anterior uveitis; Lal et al., 1999
Idiopathic Inflammatory Orbital Pseudotumors	375 mg; 3x/day x 180–660 days	8	Four patients recovered completely. One patient showed decrease in swelling, no recurrence; Lal et al., 2000
Psoriasis	1% curcumin gel	40	Decreased PhK[1] TRR[2] parakeratosis, and density of epidermal CD8+ T cells; Heng et al., 2000
Colorectal cancer	36–180 mg/day x 120 days	15	Lowered GST; Sharma et al., 2001a
Colorectal cancer	450–3600 mg/day x 120 days	15	Lowered inducible serum PGE2 levels; Sharma et al., 2004
Irritable bowel syndrome	72–144 mg/day x 56 days	207	Reduced symptoms; Bundy et al., 2004
Liver metastasis of CRC	450–3600 mg/day x 7 day	12	Low bioavailability; Garcea et al., 2004
Colorectal cancer	450–3600 mg/day x 7 days	12	Decreased M1G DNA adducts; Garcea et al., 2005
Cadaveric renal transplantation	480 mg; x 1–2/day x 30 days	43	Improved renal function, reduced neurotoxicity; Shoskes et al., 2005
Tropical pancreatitis	500 mg/day x 42 days	20	Reduction in the erythrocyte MDA levels Increased erythrocyte GSH levels; Durgaprasad et al., 2005
Ulcerative proctitis	550 mg; x 2–3/day x 60 days	5	Improved symptoms; Holt et al., 2005
Crohn's disease	360 mg; x3/day x 30 days; x 4 for 60 days	5	Improved symptoms; Holt et al., 2005

Disease	Dose; Frequency	Patient	Results; Reference
Ulcerative colitis	2000 mg/day x 180 days	89	Low recurrence; improved symptoms; Hanai et al., 2006
Familial adenomatous polyposis	480 mg; x 3/day x 180 days	5	Decrease in the number of polyps was 60.4%. Decrease in the size of polyps was 50.9%; Cruz-Correa et al., 2006
Cognitive function		1010	Better MMSE score[3]; Ng et al., 2006
PIN[4,5]		34	Rafailov et al., 2007
Helicobacter pylori infection	300 mg/day x 7 days	25	Significant improvement of dyspeptic symptoms; Di Mario et al., 2007.
Note: 1, PhK: phosphorylase kinase; 2, TRR: keratinocyte transferrin receptor; 3, MMSE: Mini-Mental State Examination Score; 4, PIN: Prostatic intraepithelieal neoplasia- 5, Zyflamend, a polyherbal preparation containing curcumin was used; Adapted from Goel et al., 2008.			

Table 10. A list of Phase I and II on-going clinical trials with curcumin

Disease	Study type	Patients	Start date
Colon cancer	Phase I, randomized	24	Completed
Colorectal cancer, ACF	Phase I, randomized[1]	-	Suspended
Colon cancer	Phase III, randomized	100	March 2006
Colorectal cancer, ACF	Phase II, non-randomized	48	September 2006
FAP	Phase II, randomized[2]	68	July 2005
FAP	Phase II, non-randomized	-	November 2005
ACF	Prevention, randomized[3]	60	April 2004
Pancreatic cancer	Phase II, non-randomized[4]	45	July 2004
Pancreatic cancer	Phase II, non-randomized	50	November 2004
Myelodysplastic syndrome	Phase II	30	-
Alzheimer's disease	Phase II, randomized	33	July 2003
Alzheimer's disease	Phase II, randomized[5]	30	Completed
Multiple myeloma	Randomized[6]	-	November 2004
Myelodysplastic syndrome	Phase I, II, non-randomized	50	December 2006
Psoriasis	Phase II, non-randomized[7]	-	October 2005
Advanced HNSCC	Phase II (1–8 g/day; 56 days)	40	-
HNSCC	Phase II/III DBRPC; (3.6 g/day, bid)	300	-
Cervical cancer (Stage IIb, IIIb)	Phase II/III DBRPC; (2 g/day, bid, 1 year)	100	-
Oral premalignant lesions	Phase II/III DBRPC; (4 g/day, bid x 28 days)	90	-
Oral premalignant lesions	Phase II/III DBRPC; (3.6 g/day, bid)	96	November 2006
Oral leukoplakia	Phase II (curcumin gel, 3x/day, 6 month)	100	-
Gall bladder cancer	Phase II (2–8 g/day)	60	-
Pancreatic cancer	Phase II (8 g/day)	40	August 2007
PSC	Phase I (8 g/day)	20	August 2007
Ulcerative colitis	Phase I (8 g/day)	20	August 2007
Barretts Metaplasia	Phase I (8 g/day)	20	August 2007
MGSU	Phase 1 (3.4 g/day)	-	-
[1]In combination with quercetin, sulindac, celecoxoib; [2]Curcuminoids; [3]NSAIDs; [4]Gemcitabine; [5]Biperine; [6]Coenzyme Q10; [7]Curcumin C3 Complex®; ACF = aberrant crypt foci; DBRPC= double-blind randomized placebo-controlled; HNSCC = head and neck squamous cell cancer; MGSU = Monoclonal Gammopathy of Unknown Significance; FAP = Familial adenomatous polyposis. Majority of these studies are cited in National Cancer Institute clinical trial database.			

2.9. Pharmacological effects

The pharmacological effects of curcumin and turmeric have been investigated in multiple studies. As these studies are not expressly related to safety, a brief overview of some of these studies is presented in Table 9. The pharmacological actions of curcumin include functioning as an antioxidant, as an antiinflammatory, anti-cancer, and possessing antiatherogenic effects. Due to these properties, the National Cancer Institute, Chemoprevention Branch has considered curcumin for clinical development (Anonymous, 1996). In some studies, curcumin has been reported to induce some forms of detoxifying enzymes while inhibiting other forms involved in the activation of carcinogens. Curcumin has been reported to increase the levels of glutathione by over 40% in rat liver cells *in vitro* at non-cytotoxic concentrations (White et al., 1998; Piper et al., 1998; Singh, et al., 1998). Glutathione along with the phase II metabolic enzyme glutathione-S-transferase is involved in the metabolism of chemical substances via conjugation. The available information indicates that the pharmacological effects of curcumin are unlikely to affect its safety profile.

2.10. Miscellaneous Studies

In a recent study, Tang et al. (2008) reported that the oxalate content of turmeric may increase the risk of kidney stone formation in susceptible individuals. These investigators quantified the total and soluble oxalate content of turmeric and cinnamon samples. The total oxalate content of cinnamon and turmeric, analyzed in duplicate on 4 occasions, was 1789±54 and 1969±56 mg/100 g, respectively. The percentage of oxalate that was water soluble differed markedly between cinnamon (107±8 mg/100 g, 6% of total) and turmeric (1788±1 mg/100 g, 91% of total). In a human study, these investigators compared the change in urinary oxalate excretion following turmeric and cinnamon exposure. In this 8-week randomized, cross over trial, eleven healthy subjects (21-38 year-old) ingested supplemental doses of cinnamon (n=6) and turmeric (n=5) for 4-wk periods that each provided 55 mg oxalate/day. Oxalate load tests, which entailed the ingestion of a 63 mg dose of oxalate from the test spices, were performed after each 4 week experimental period and at the study onset with water only (control treatment). Urine analysis of the subjects receiving turmeric and cinnamon revealed a significantly higher computed 6 hour oxalate absorption rate from turmeric ingestion (8.2%) than from cinnamon ingestion (2.6%), which was most likely explained by the 91% soluble oxalate content of turmeric compared with 6% for cinnamon. The water soluble oxalate noted in the analytical experiments appeared to be the primary cause of the greater urinary oxalate excretion/oxalate absorption from turmeric. The investigators suggested that consumption of supplemental doses of turmeric, but not cinnamon, can significantly increase urinary oxalate levels, thereby increasing the risk of kidney stone formation in susceptible individuals.

Because of the concerns raised by the Tang et al. (2008) study, Sabinsa Corporation analyzed samples of Curcumin C3 Complex® for the presence of oxalate (Sabinsa 2009). In this analysis, the amount of oxalic acid detected in Curcumin C3 Complex® was 250 ppm (0.025%). Tang et al. (2008) reported that 2.8 g turmeric (the dose used in their study) provided 55 mg oxalic acid (~90% water soluble). This is equivalent to 20 mg of oxalic acid/g of turmeric (or 20,000 ppm). Thus the oxalic acid content of turmeric used in Tang et al. (2008) study is over 80-fold higher than that found in Curcumin C3 complex®. The intended food uses of Curcumin C3 complex® in specified food categories will result in the 90th percentile intake of 180 mg of Curcumin C3 Complex®. As Curcumin C3 Complex® contains 250 ppm oxalic acid; the resulting

90th percentile intake of oxalic acid will be 0.045 mg. According to the Oxalosis & Hyperoxaluria Foundation (OHF, 2008), a high oxalate food is classified to have 22-99 mg oxalic acid per serving, while a low oxalic acid food is classified to contain 5-10 mg oxalic acid per serving. The intended uses of Curcumin C3 Complex® will result in ~0.045 mg of oxalic acid which is very small (489-fold lower) or insignificant as compared to the foods that are considered as high oxalate food. The available information demonstrate that oxalic acid intake from consumption of Curcumin C3 Complex® is safe.

3. SUMMARY

Curcumin is the characteristic yellow coloring component of the popular Indian curry spice turmeric. While the term curcumin is commonly used to represent all the curcuminoids found in the extract, curcumin is the principal curcuminoid found in turmeric. The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. As a spice, turmeric and its oleoresin are used to provide curry with its distinctive yellow color and flavor. It is also used as a coloring agent in cheese, butter, and other foods. Curry powder which may contain varying levels of curcumin (up to 30%) is used in several foods. The United States is the largest consumer of the curcumin oleoresins. There is also a long history of food use for both turmeric and curcumin suggesting that repetitive human exposure to curcumin has been without reports of significant adverse effects. Use of turmeric, turmeric extract, or turmeric oleoresin in foods for human consumption is considered as GRAS by FDA and FEMA and a permanent ADI of 0-3 mg/kg bw /day has been previously established by the JECFA. In addition to its uses as dietary spice and coloring agent, turmeric has a long history of use as therapeutic agent for numerous health conditions. The ancient texts of the Indian traditional health care system, Ayurveda describe the use of curcumin for a wide variety of health benefits.

Sabinsa Corporation intends to use curcumin (Curcumin C3 Complex®) prepared by solvent extraction of turmeric using acetone or ethyl acetate as a flavoring agent at use levels of 20 mg/serving in Baked Goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors.. Curcumin C3 Complex®, manufactured according to current good manufacturing practices, contains >95% curcuminoids of which between 70 to 80% is curcumin. The mean and 90th percentile all-user intake of Curcumin C3 Complex® from its intended food uses is estimated as 97.44 and 180.25 mg/person/day, respectively. The estimated 90th percentile intake (~180 mg/day) of Curcumin C3 Complex® resulting from the intended use levels in specified foods is equivalent on a body weight basis to ~3 mg/kg bw/day for an adult weighing 60 kg.

In two separate subchronic studies, dietary exposure of rats to curcumin at doses up to 2% or at 10% for 8 weeks did not reveal any adverse effects. The decrease in food intake and subsequent lower feed efficiency noted at the highest dosage (10% curcumin) was attributed to effects of curcumin on food palatability. In 90 day studies (NCI Chemoprevention Branch), the NOELs in dogs and rats were found to be greater than 1000 mg/kg/day and 3500 mg/kg/day, respectively, the highest dose studied. In studies in rats and dogs, minor changes in body weights in rats and in hematological values in rats and dogs were not considered to be biologically significant. In 90-day feeding studies in rats and mice, the no adverse effect level with respect to gross and microscopic pathological changes was 5% of the diet (2587 and 2760 mg/kg/day in male and female rats and 7700 and 9280 mg/kg/day in male and female mice, respectively). Thus, there is over a 800-fold safety factor from the NOEL in rats and a 2400-fold safety factor

from the NOEL in mice compared to the intake from the proposed intended uses of Curcumin C3 Complex®.

In the NTP rodent chronic studies evidence of hepatotoxicity was noted, but pharmacokinetic studies suggest differential metabolism of curcumin in humans and rodents. It appears that rodents might be particularly susceptible to curcumin effects on the liver. In carcinogenicity studies in rats and mice, turmeric oleoresin containing 79 to 85% curcumin produced equivocal responses. The JECFA reviewed the NTP studies and concluded that although increases in the incidences of hepatocellular adenomas, small intestinal carcinomas and pituitary gland adenomas in mice and clitoral gland adenomas (females) in rats were noted, the effects were not dose-related, and that curcumin was not a carcinogen (WHO, 1996).

In genotoxicity studies, curcumin was not mutagenic in the Ames assay or in the mouse dominant assay in the presence or absence of metabolic activation. In the *B. subtilis* Rec assay, growth inhibition due to DNA damage was noted following exposure to curcumin. In gastric mucosa cells and peripheral blood lymphocytes, curcumin caused DNA damage in the Comet Assay. However, damaged cells were able to recover within a period of 2 hours. In *in vitro* and *in vivo* clastogenicity assays, equivocal results have been reported. 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 in assays for the induction of chromosomal aberrations. Overall, there was no evidence to show that curcumin was genotoxic.

In a two-generation reproductive toxicity study in rats, no adverse effects of curcumin at levels of up to 10,000 ppm (1%) in diet were noted. The study was conducted according to OECD guidelines and generally the guidelines recommend use of 1% as limit dose. In this study, the NOAEL for reproductive toxicity of curcumin was determined as 10,000 ppm, which is equivalent to 847 and 959 (for male rats) and 1043 and 1076 mg/kg bw/day (for females) for F0 and F1 generations, respectively. This study was also reviewed by JECFA and considered that the small body weight reduction in the F2 pups of the highest dose group prevented the highest dose from being regarded as a no adverse effect level, and hence JECFA allocated an ADI for curcumin of 0–3 mg/kg based on the intake of 250–320 mg/kg in the mid-dose group (3000 ppm) as the NOEL. A critical review of the multigenerational study suggest a more appropriate and reasonable NOAEL, also determined by the authors of the study, of 847 - 1076 mg/kg body weight/day, the highest dose tested. Comparing this dose to the 90th percentile intake of Curcumin C3 Complex® there is over a 270-fold safety factor compared the reproductive study.

Available clinical studies showed that curcumin generally had no adverse effects and curcumin is not toxic even at very high doses. Ingestion of curcumin doses of approximately 2000 mg/day for 120 days was without adverse effects. Results from a Phase I clinical trial in which no evidence of toxicity was seen in 25 subjects using up to 8000 mg/day of curcumin for 3 months provide an ample margin of safety above the resulting intake of 180 mg/day (3 mg/kg bw/day) of Curcumin C3 Complex® from its intended uses. Both JECFA and EFSA panel have concluded that the available evidence supports an ADI of 3 mg/kg bw/day for curcumin. The most compelling and key rationale for the continuing traditional use of curcumin for its potential health benefits is its extremely good safety profile.

The clinical evidence of Curcumin C3 Complex® safety is supported by:

- Both turmeric and curcumin have a long history of food use.

- Humans have been regularly exposed to curcumin without reports of significant adverse effects. Turmeric is considered GRAS by FDA.
- In multiple human clinical studies, the safety of curcumin was determined at doses up to 8000 mg/day.
- There is no evidence that consumption of curcumin either in foods or as a dietary supplement has any cumulative effect.
- Experimental studies, including subchronic toxicity, chronic toxicity and carcinogenicity, reproduction and developmental toxicity, and *in vitro* and *in vivo* genotoxicity findings corroborate the human clinical safety data.

There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use or acceptable daily intake (ADI) for Curcumin C3 Complex®. The safety determination of Curcumin C3 Complex® is based on several lines of evidence, including human clinical trials, and a variety of animal studies. The totality of available evidence supports the safety of Curcumin C3 Complex® at the 90th percentile intake of 180 mg/person/day. On the basis of scientific procedures[3], and history of exposure from natural dietary sources, the consumption of Curcumin C3 Complex® as an added food ingredient is considered safe at use levels up to 20 mg/serving. The intended uses are compatible with current regulations, *i.e.,* Curcumin C3 Complex® is used in specified foods (described in this document) and is produced according to current good manufacturing practices (cGMP).

[3] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that Curcumin C3 Complex®, meeting the specifications cited herein, and when used as a flavoring agent (flavor enhancer) [21 CFR§170.3(o)(11)] and as an antioxidant at maximum use levels of up to 20 mg/serving (when not otherwise precluded by a Standard of Identity) in specific foods (Baked Goods; Soups; Snack Foods; Imitation Dairy Products; and Seasonings & Flavors) described in this monograph and resulting in the 90th percentile all-user estimated intake of 180 mg Curcumin C3 Complex®/person/day is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Curcumin C3 Complex®, when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

John A. Thomas, Ph.D., F.A.T.S. — Date: 2/1/13

(b) (6)

Stanley M. Tarka, Jr., Ph.D. — Date: 31 January 2013

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.T.S. — Date: 4 February 2013

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6. APPENDIX I

Analytical data from different manufacturing lots prepared using acetone or ethyl acetate (Sabinsa, 2012)

Appendix I-A: Specifications of Curcumin C3 Complex® from five different batches prepared using acetone as extraction solvent

Parameter	Standard*	Lot # C61656	Lot # C71972	Lot # C61624	Lot # C81655	Lot # C81423
Description	Crystalline powder	Complies	Complies	Complies	Complies	Complies
Identification	HPLC	Complies	Complies	Complies	Complies	Complies
Loss on drying	NMT 2%	1.62	0.4	1.01	0.37	0.77
Ash content	NMT 1%	0.09	0.05	0.16	0.02	0.01
Melting range	Melts between 172°C to 178°C	172°C to 178°C	174°C to 174°C	172°C to 174°C	173°C to 176°C	176°C to 178°C
Tapped bulk density	Between 0.50 and 0.90 g/ml	0.5	0.59	0.63	0.56	0.56
Loose bulk density	Between 0.30 and 0.50 g/ml	0.3	0.4	0.33	0.39	0.40
Sieve test (passes through)						
- 20 mesh	NLT 100%	100	100	100	100	100
- 40 mesh	NLT 95%	99.25	99.79	99.82	98.04	100
- 80 mesh	NLT 75%	96.97	94.7	98.02	89.76	99.91
Assay						
Total curcuminoids	NLT 95% on dry basis	95.46	95.86	95.92	95.65	96.13
Purity of curcuminoids						
Bisdemethoxy curcumin	NLT 2.2% and NMT 6.5%	2.9	2.9	4.44	2.55	3.69
Demethoxy curcumin	NLT 15% and NMT 22%	15.17	17.9	19.5	19.85	20.77
Curcumin	NLT 75% and NMT 81%	79.80	79.18	76.06	77.59	75.33
Heavy metals		10	10	10	10	10
Arsenic	1 ppm	0.2	0.59	0.2	<0.2	<1
Lead	<2 ppm	0.2	1.82	0.2	0.76	0.75
Microbiological assays						
Total plate count	< 5000 cfu/g	<100	<100	<100	<100	<100
Yeast and Mold	< 100 cfu/g	< 10	<10	<10	<10	<10
Escherichia coli	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
Salmonella	Negative (cfu/10 g)	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Negative (cfu/10g)	Absent	Absent	Absent	Absent	Absent
Coliform	≤3/g	≤3	≤3	≤3	NA	≤3

*Standard specifications for marketed product; **Heavy metal analysis performed by AAS or ICP-OES method. NA = Not available.

Residual solvent levels from different batches of Curcumin C3 Complex prepared using acetone		
Batch No.:	**Isopropyl alcohol (ppm)**	**Acetone (ppm)**
C90906	ND	5.63
C90907	6.1	34.0
C90909	ND	15.80
C91081	ND	22.16
C91099	2.32	16.15

Residual solvent levels in compliance with USP

Appendix I-B: Specifications of Curcumin C3 Complex® from three different batches prepared using ethyl acetate as extraction solvent

Parameter	Standard*	Lot # C111874E	Lot # C111949E	Lot # C111950E
Description	Crystalline powder	Complies	Complies	Complies
Identification	HPLC	Complies	Complies	Complies
Loss on drying	NMT 2%	0.32	0.38	0.59
Ash content	NMT 1%	0.15	0.20	0.05
Melting range	Melts between 172°C to 178°C	NA	NA	NA
Tapped bulk density	Between 0.50 and 0.90 g/ml	0.64	0.59	0.56
Loose bulk density	Between 0.30 and 0.50 g/ml	0.37	0.36	0.35
Sieve test (passes through)				
- 20 mesh	NLT 100%	100	100	100
- 40 mesh	NLT 95%	99.80	99.80	99.85
- 80 mesh	NLT 75%	99.10	99.50	99.69
Assay				
Total curcuminoids	NLT 95% on dry basis	95.83	95.15	95.46
Purity of curcuminoids				
Bisdemethoxy curcumin	NLT 2.2% and NMT 6.5%	3.15	2.50	2.50
Demethoxy curcumin	NLT 15% and NMT 22%	18.94	18.09	18.09
Curcumin	NLT 75% and NMT 81%	78.12	79.41	79.41
Heavy metals		10	10	10
Arsenic	1 ppm	0.40	<0.2	0.28
Lead	<2 ppm	0.24	<0.2	<0.2
Microbiological				
Total plate count	< 5000 cfu/g	<100	<100	<100
Yeast and Mold	< 100 cfu/g	< 10	<25	<10
Escherichia coli	Negative (cfu/10g)	Absent	Absent	Absent
Salmonella	Negative (cfu/10 g)	Absent	Absent	Absent
Staphylococcus aureus	Negative (cfu/10g)	Absent	Absent	Absent
Pseudomonas aeruginosa	Negative (cfu/10g)	Absent	Absent	Absent

*Standard specifications for marketed product; **Heavy metal analysis performed by AAS or ICP-OES method. NA = Not available.

Residual solvent levels from different batches of Curcumin C3 Complex prepared using ethyl acetate		
Batch No.:	**Isopropyl alcohol (ppm)**	**Ethyl acetate (ppm)**
C111874E	**8.00**	43.00
C111949E	10.00	40.00
C111950E	9.00	42.00

Residual solvent levels in compliance with USP

SUBMISSION END