Accepted Manuscript

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PII: S0273-2300(17)30002-8

DOI: 10.1016/j.yrtph.2017.01.002

Reference: YRTPH 3749

To appear in: *Regulatory Toxicology and Pharmacology*

Received Date: 21 December 2016

Accepted Date: 17 January 2017

Please cite this article as: Yates, A., Erdman Jr., , J.W., Shao, A., Dolan, L., Griffiths, J.C., Bioactive nutrients - Time for tolerable upper intake levels to address safety, *Regulatory Toxicology and Pharmacology* (2017), doi: 10.1016/j.yrtph.2017.01.002.

Bioactive Nutrients - Time for Tolerable Upper Intake Levels to Address Safety

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KEYWORDS

Bioactives, Nutrition, Tolerable Upper Intake Levels, Dietary Reference Intakes, DRIs, Nutrient Reference Values, Lutein, Green Tea, EGCG, Epigallocatechin gallate

INTRODUCTION

There is increasing interest by consumers, researchers, and regulators into the roles that certain bioactive compounds, derived from plants and other natural sources, can play in health maintenance and promotion, and even prolonging a productive quality of life. Research has rapidly emerged suggesting that a wide range of compounds and mixtures in and from plants (such as fruits and vegetables, tea and cocoa) and animals (such as fish and probiotics) may exert substantial health benefits. There is interest in exploring the possibility of establishing recommended intakes or dietary guidance for certain bioactive substances to help educate consumers. A key aspect of establishing dietary guidance is the assessment of safety/toxicity of these substances. Toxicologists need to be involved in both the development of the safety framework and in the evaluation of the science to establish maximum intake/upper limits.

MODELS FOR ESTABLISHING UPPER LEVELS

Possible approaches to determining safety of dietary bioactive components are those used to establish upper intake levels for nutrients (IOM, 1998a). Initiated by the Food and Nutrition Board (FNB) in 1994 for the United States and Canada, the development of Dietary Reference Intakes (DRIs) for nutrients though 2004 included not only recommended dietary intakes (RDAs) as had been issued since 1941, but also introduced Tolerable Upper Intake Levels (ULs) for nutrients, applying risk assessment methodology. This approach followed reports from the United Kingdom in 1991 (COMA, 1991) and from ILSI in 1994 (Mertz, et al.,

1994) which identified the need for upper reference values due to the increased use of fortified foods and availability of dietary supplements, permitting nutrient intakes to exceed that typically obtained from natural foods alone.

The DRI process as envisioned by the Food and Nutrition Board in 1994 (IOM, 1994) not only included reviews of known nutrients, but also reviews of the literature to establish reference values for other food components, now termed *bioactives*, wherever possible. While past FNB RDA reports focused on amelioration of deficiency conditions, the DRI process was to also include endpoints related to decreasing risk of chronic disease. While this had been the plan, over the 10 years of the DRI process, reference values were only developed for one bioactive compound class evaluated, fiber (IOM, 2002).

The DRI Upper Level model as developed draws heavily on toxicology tenets that must be tweaked since a nutrient, unlike most additives and contaminants, has a minimum level of intake that is required to maintain health. The definition of the UL focuses on adverse health effects in the general population. A rotating subcommittee composed of toxicologists and nutritionists developed and reviewed all the published data over the 10-year period to develop ULs for 24 of the 37 vitamin and mineral nutrients reviewed. The UL is based on either a *No Observed Adverse Effect Level (NOAEL)* or a *Lowest Observed Adverse Effect Level (LOAEL)*, and then decreased by dividing by a factor based on the uncertainty of how applicable to the entire population the available data are, and the seriousness of the known adverse effects. The process for developing ULs based on nutrient risk assessment is now globally accepted as the

64 approach for establishing upper level reference values and regulatory maximums and is used in
65 US/Canada, Europe, China, Southeast Asia and some Latin American markets.

66 Aspects to consider when applying the DRI UL method to bioactive components are the
67 extent of data regarding intakes of bioactive components and documented adverse effects, and
68 available estimates of typical dietary intakes of the substances in the population. While
69 detailed food composition databases are available for nutrients (e.g., USDA Nutrient Database,
70 www.ndb.nal.usda.gov), such databases for content of bioactive components in foods are in
71 their infancy. In addition, many bioactive components with possible health benefits are groups
72 of chemical compounds within foods (such as flavonoids), rather than easily identifiable single
73 substance such as a vitamin or mineral.

74 While there is an idealized benefit/risk curve for nutrients, there may be overlapping
75 distributions in a population where the amount needed to obtain maximum benefit for one
76 individual may be greater than the amount that may result in an adverse effect due to excess
77 for another, or the adverse effect in the same individual overlaps with the amount needed for
78 benefit; for example, the effect of increasing fluoride intake to decrease dental caries overlaps
79 with the increasing incidence of dental fluorosis or mottling (IOM, 2007).

80 While there can be a number of adverse effects associated with high intakes of a
81 nutrient, the UL is based on a specifically defined adverse effect that would be most
82 detrimental to the population. For example, for folate, the adverse effects reported in the
83 literature prior to 1998 when the DRI review was done included a) neurological damage in
84 vitamin B12-deficient individuals, b) general toxicity as found in mental changes, sleep

disturbances, and GI effects at 15 mg/day, c) increased cancer of oropharynx and hypopharynx and total cancer rates in an epidemiological study, and d) hypersensitivity, which was rare, at 1 mg/day (IOM, 1998b). At the time there were about 100 reported cases of neurological damage with supplemental folate consumed at ≥ 5 mg/day, while there were only 8 well-documented cases at < 5 mg/day. Based on this LOAEL, the Uncertainty Factor (UF) was chosen as 5, due to the severity of the neurological complications and their irreversibility. However, it was not higher than 5 because there were uncontrolled observations in millions of people taking 1/10th the LOAEL of 5 with no reported harm. Similar DRI UL reviews were done for all 37 vitamins and minerals evaluated.

Since the DRI reports were released beginning in 1997, other groups have undertaken in-depth risk assessments of nutrients for upper levels using similar methodologies. The most extensive were conducted by the European Union Scientific Committee on Foods (2000, 2002) subsequently now under the European Food Safety Authority (EFSA, 2004) and the United Kingdom's Expert Group on Vitamins and Minerals (EVM, 2003). Not surprisingly, resulting ULs have differed, even when using the same datasets, due to different choices of adverse effects upon which to base a UL, and different UFs based on committee consensus. A comparative analysis of the three approaches (DRI, EVM, and UK) has been published (IOM, 2007).

Other possible reference value approaches have been proposed. One approach proposed in 2006 at the FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006) for use when there is little NOAEL or LOAEL data upon which to conduct a risk assessment is to establish the Highest Observed Intake (HOI), derived only when no adverse

health effects have been identified. The HOI is the highest level of intake observed (or administered as reported within a study of acceptable quality); this could be the 90th or 95th percentile of estimated intakes in a population with no apparent adverse effects. However, it is important that the HOI should be overtly differentiated from the UL to prevent its misinterpretation or use.

The FAO/WHO report also highlighted the critical issues faced when developing ULs for nutrients: that nutrient substances are subject to complicated homeostatic mechanisms that may control and alter absorption, utilization, storage, and/or transport which may typically not occur with contaminants or additives, and that there are few valid *causally* associated biomarkers that are known surrogates for adverse effects. Thus the likelihood of being able to establish an UL based on risk assessment, particularly for bioactive components in the diet which are less well characterized, becomes quite difficult. Long-term or habitual intake data are required to determine both the relation between the biomarker and adverse effect and to characterize risk. Thus the HOI could provide guidance on where to limit intake for substances such as bioactives when valid risk assessments can't be obtained.

APPLICATION OF TOXICOLOGY DECISION-MAKING

The main steps involved in developing tolerable upper intake levels (ULs) are 1) identification of the critical effect, 2) determination of the point of departure (POD) of the dose response curve, and 3) application of appropriate uncertainty factors (UFs) to the POD. Although risk assessors often focus on the second and third points, identification of the critical

effect is of utmost importance, as an UL predicated on a non-critical effect may not protect the consumer against toxicologically relevant effects.

To determine the critical effect of a food or dietary supplement ingredient, risk assessors should review studies with oral exposure. Human data are preferable to animal data and intervention studies (particularly randomized, double blind, placebo controlled) are more useful than observational. Information from animal species whose biological responses are most similar to humans is more valuable than other animal data, but usually studies in rats or mice, which may not be the best models are used to derive an UL when reliable human data are not available.

Lewis and coworkers (2002) provided three pointers to help toxicologists select the data set that identifies the critical endpoint. First, *is there a difference compared to control?* Usually this is identified by an appropriate statistical analysis. Second*, is the difference an effect of treatment?* A difference is more likely to be an effect of treatment if there is an obvious dose-response relationship. The difference should not be due to inclusion of statistical outliers, and the value should not be within the range of historical controls. A valid method should be used to measure the endpoint that changed and the effect should be biologically plausible. A difference is less likely to be an effect of treatment if there is a difference from a baseline measurement but not from a concurrent control or if the result contradicts any of the points mentioned above that are used to identify an effect of treatment.

The third question posed by Lewis is *is the effect adverse?* An effect is more likely to be adverse if it is outside of the normal range, irreversible and affects the performance of the

148 whole organism or reduces an organism's ability to respond to an additional change. An effect
149 is less likely to be adverse if it is a consequence of the experimental model, if there is no
150 alteration in the general function of the test organism or organ/tissue affected, if the effect is
151 not part of a continuum of changes known to progress with time to an established effect, or if
152 the effect is transient, of limited severity, isolated or independent, secondary to other adverse
153 effects or adaptive (Lewis et al., 2002). Some thought needs to be put into whether an effect is
154 adaptive or adverse, as some adaptive responses (e.g. enzyme induction) could potentially be
155 adverse.

156 When evaluating animal data to uncover a potential critical effect, key events in the
157 animal mode of action must be plausible in humans. If they are not plausible, the mode of
158 action may be specific for the animal and not relevant for humans. Further, if the key event in
159 animals is not plausible in humans due to differences in toxicokinetics or toxicodynamics, it is
160 also not relevant for humans (Cohen et al., 2004). For example, if a substance of interest has
161 been identified as a possible or probable human carcinogen from a rodent carcinogenicity
162 study, it is still possible to derive an UL for humans from other data if a successful argument for
163 why the carcinogenicity study was not appropriate for humans can be made, particularly if the
164 tumor response is due to excessive dietary exposure or has no human correlate.

165 In recent years, the concept of adverse outcome pathway (AOP) has been promoted as
166 a means to link key molecular and cellular events to adverse outcomes (Ankley *et al.*, 2010). If a
167 response in an AOP is found in a study, the pathway can be followed to the ultimate
168 toxicological event. AOPs must be validated and currently there are over 100 AOPs in the

process of validation. While DRI ULs were not identified for some nutrients (e.g. chromium, vitamin K, thiamin, riboflavin, vitamin B12, pantothenic acid and biotin), it is altogether possible that AOPs could be used to help identify ULs for these substances in the not too distant future.

The POD of the dose response curve may be identified using the no observable adverse effect level (NOAEL), lowest observable adverse effect level (LOAEL) or the benchmark dose (BMD). There are advantages and disadvantages to each approach; however, the BMD offers some clear advantages over the NOAEL or LOAEL. BMDs take the shape of the dose-response curve into account and are not limited by tested doses, there is flexibility in determining biologically significant rates, and dichotomous or continuous data may be analyzed.

Uncertainly factors (UFs) are usually applied to the POD to derive safe doses of food ingredients for humans. UFs for various extrapolations (e.g. interspecies, intraspecies, subchronic to chronic, LOAEL instead of NOAEL, and database adequacy) range from 1- 10, with 10 as the default value. In theory, a UF of 100,000 could be applied to a LOAEL value from a 90-day rat study. Typically, a default UF of 100 is applied to the NOAEL from a 90-day guideline study in rats to derive the safe dose of a food ingredient for humans unless data support use of a lower or higher UF. The default UF of 100 is the product of default UFs of 10 each for interspecies and intraspecies differences in toxicodynamics and toxicokinetics (WHO, 2005). Derived UFs for toxicokinetics can be calculated using clearance, area under the curve (AUC), or maximum blood, serum or plasma concentration (Cmax) data. Clearance data should be normalized to body weight and AUC or Cmax data should be normalized to dose. Calculating a

189 UF for toxicodynamics involves comparing doses that cause a defined change in a key endpoint
190 (usually 10%). *In vitro* studies or the BMD may be useful for this calculation.

191 In general, when developing ULs for nutrients, the IOM used UFs for intraspecies
192 extrapolations that were much lower than the default value of 10, even if a LOAEL was used as
193 the POD. The UFs used by the IOM for rat to human extrapolations were also relatively low, as
194 exemplified by the cases of vitamin E and molybdenum (IOM 2000, 2001). The UF for vitamin E
195 relied on a rat LOAEL from a 13-week study and used UFs of 2-3 for various extrapolations
196 because of the availability of data showing that animals and humans have similar responses to
197 the vitamin.

198 Different agencies may use different data sets or approaches to develop ULs or similar
199 reference values for the same substance. The IOM used a relatively small study in women and
200 applied a UF of 1.5 to the POD to develop an UL of 40 mg/day for zinc in adults (IOM, 2001).
201 The UL for 4-8 year olds was derived using data from a study in infants, scaling up for body
202 weight. In contrast, the EC Scientific Committee (2003) applied a UF of 2 to the POD from three
203 human studies to derive an UL for adults of 25 mg/day and used the same data to derive the UL
204 for 4-8 year olds, scaling down for body weight. JECFA has established acceptable daily intakes
205 (ADIs) for some food additives that are bioactive, for example curcumin and lycopene (EFSA,
206 2008, 2010). The ADIs for these substances were derived by applying UFs of 100 to PODs from
207 long term rat studies (a multigenerational study for curcumin and a one year study for
208 lycopene), showing that JECFA was very conservative when setting ADIs.

To conclude, Identification of the relevant critical effect from the dataset is of utmost importance in deriving an UL. The science of toxicology is evolving to identify the critical effect at the molecular level, which may help identify the basis for setting ULs for some substances. The goal is to derive an UL that is protective of the population but not unduly restrictive and different approaches can be taken to derive the value. The BMD offers certain benefits over the NOAEL for the POD, and default UFs do not have to be applied to the POD if they can be refined using experimental or modeled data.

LUTEIN: A BIOACTIVE CASE STUDY

Traditionally, RDAs were developed to establish dietary levels for essential nutrients to prevent development of symptoms of nutrient deficiency diseases (IOM, 1994). Subsequently, the DRI approach was envisioned to additionally consider non-essential food components as well as chronic disease endpoints (IOM, 1994). Essentiality of a food component implies that removal of that component from the diet results in adverse symptoms, which are then reversed when that component is added back to the diet. Among the 42 DRI nutrients for which recommended intakes were established, not all of them meet the classical criteria to be considered essential. Fiber, fluoride, and perhaps choline, were not considered essential based upon the available human clinical data, but have DRIs based on their roles in health. Are there other dietary bioactive components that might be considered to play important roles in health but don't meet the classical definition of essential?

While reviewed as a part of the DRI process in 2000 (IOM, 2000) with no resulting reference values being established, a case for considering lutein for DRI status now can certainly be made. Age-related macular degeneration (AMD) is a chronic disease resulting in impaired vision and blindness in all too many older Americans. In fact, it is the number one cause of blindness in persons over 65 years of age. The dietary component, lutein, along with zeaxanthin, deposits in the macular fovea pit and is associated in many clinical trials with increased macular pigment optical density (MPOD), which in turn is associated with reduced risk of AMD. Elevated MPOD is also associated with improved visual performance, visual acuity, and glare sensitivity (Biesalski et al, 2013).

Lutein is a carotenoid found in green leafy vegetables, corn, eggs, avocados, and other fruits and vegetables. It is one of the primary carotenoids in human blood and tissues, and remarkably lutein concentrations in the central retina are > 500-fold higher than concentrations in other body tissues (Biesalski et al, 2013). Specific binding proteins for both lutein and zeaxanthin are located in the macula to facilitate their substantial deposition there, thus supporting the concept that there is a purpose for doing so. As it does in chloroplasts in green plants, lutein appears to play a role in protection of the retina from excess light damage and risk of oxidative damage to surrounding tissue.

In order for bioactive components to qualify for evaluation through a DRI-like process, Lupton et al (2014) proposed a 9-pointset of criteria which includes the following: 1) definition of the substance, 2) established and validated methods to analyze the compound in foods, 3) a database of amounts in foods, 4) prospective cohort studies, 5) clinical trials, 6) dose response

data, 7) systematic reviews, 8) biological plausibility, and 9) safety data. Since there is published data to support each of these criteria for lutein, it can be concluded that this carotenoid is ready for evaluation (Wallace et al, 2015).

Safety of lutein

Based on the available evidence, enhancement of MPOD by increasing lutein intake is associated with a decreased risk of AMD, providing a foundational argument for the essentiality of lutein. There are additional, on-going clinical trials with lutein supplementation that are probing specific lutein eye function interrelationships. Outcomes from these studies and other work with non-human primates should shed more light on the essentiality of lutein. One of the evaluation criteria necessary in consideration of potential DRI-like recommendations for lutein is its safety when consumed from foods or from supplements.

Carotenodermia is defined as the presence of carotenoids (which include alpha and beta carotene, lycopene lutein, and zeaxanthin) in the skin – usually an orange discoloration in the palms of the hands and soles of the feet and other skin areas. This is commonly seen in children and in some vegetarians. It is harmless although it may take months for the color to fade upon reduction of intake of foods or supplements high in carotenoids. Shao and Hathcock (2006) reviewed the safety of lutein and found no adverse events mentioned in 30 peer-reviewed studies involving lutein, other than carotenodermia.

Carotenodermia has been reported in some trials where lutein supplements of 15 mg daily were consumed for 4 – 5 months. Lutein doses in clinical trials ranged as high as 40 mg/day for nine weeks followed by an additional 17 weeks at 20 mg/day. More recently the

AREDS2 trial was completed where some subjects received 10 mg/day of lutein plus 2 mg/day of zeaxanthin for 5 years (Chew et al, 2015) with no adverse events from the lutein-zeaxanthin supplement reported. Placed into context, the average lutein consumption by Americans from foods is estimated to be less than 2 mg/d (Johnson, 2014).

Shao and Hathcock (2006) developed a he Highest Observed Intake (HOI) as established by FAO/WHO (2006) as well as an Observed Safe Level (OSL) as proposed by Hathcock (2004); these would appear to be appropriate assessment methods to set safety levels for lutein. They concluded that evidence of safety is strong at intakes of up to 20mg/d; thus this level could serve as the OSL or HOI. Theirs was the last published systematic evaluation of the safety of lutein; although a decade old, no additional adverse safety concerns have emerged. .

It would appear that a new evaluation of lutein for DRI-like recommendations is appropriate. While using the established DRI framework may not be best for bioactive food components such as lutein, the 9-point criteria suggested by Lupton and co-authors (2014) is justified. There are data for all 9 criteria, including for safety at dietary levels that would be anticipated to be important to reduce the risk of AMD.

DEVELOPMENT OF AN UL FOR A BIOACTIVE; EGCG: FRIEND OR FOE?

Tea is the most commonly consumed beverage in the world, with total annual sales exceeding $43 billion globally, more than $11 billion of which is accounted for by green tea (Euromonitor, 2015). A growing body of evidence continues to emerge demonstrating a variety

of potential health benefits from consumption of green tea and its constituents (Cassidy et al., 2015; Jacques et al., 2013; Peng et al., 2014). Indeed, these health benefits have led to or been part of a range of discussions focused on the prospect of establishing dietary guidance or even recommended intakes for tea and/or tea constituents (Gaine et al., 2013; Lupton et al., 2014; Wallace et al., 2015).

However, simultaneously, green tea, in particular concentrated green tea extracts (GTE), have been the subject of safety concerns. Green tea catechins, including the well-known constituent epigallocatechin gallate (EGCG), have been implicated in both the benefits (Legeay et al., 2015; Fujiki et al., 2015) and harms (Blumberg et al., 2015; Harrison-Dunn, 2016) from green tea. Many of the safety concerns stem from published case reports asserting a link between concentrated GTE consumption and liver injury (Harrison-Dun, 2016; Teschke et al., 2014). This suggests a “risk-benefit” curve applies similar to that with essential nutrients (Murphy et al., 2016), and has led some European regulatory agencies to establish or propose daily EGCG limits (to be applied to supplements). These values vary widely, with little or no scientific basis or rationale provided. For example, France initially proposed a 35 mg/day limit (MEF, 2012), then later modified this to 300 mg/day (OJFR, 2014). Italy established a similar 300 mg/day limit (IMOH, 2016), while Belgium established a 1600 mg/day limit (BOJ, 2012). In 2009, the European Food Safety Authority (EFSA) Scientific Cooperation Project (ESCO) published a safety assessment on green tea, focusing on dried extracts and traditional infusions used as food including beverages and food supplements in the EU, but a specific UL for EGCG was not proposed (EFSA J, 2009).

Establishing an EGCG tolerable upper intake level (UL) based on risk assessment could have a number of benefits. The current lack of a science-based limit causes confusion and promotes an overly conservative approach by some governments. Industry, regulators and practitioners all need appropriate guidance on what constitutes an appropriate limit (if any) of GTE.

Methods

The aim of the present analysis was to conduct an EGCG risk assessment using the basic principles of nutrient risk assessment to establish a proposed EGCG UL as described in the FAO/WHO Technical Report (FAO/WHO, 2006). The basic methodological approach involved evaluation of three main data sets: animal toxicology data, human intervention studies, and published case reports and publicly available adverse event reports. For all three data sets searches were conducted in the PubMed database, including peer-reviewed studies published in English through May 2016. Search terms included “green tea”, “green tea extract”, “catechins”, “flavan-3-ols” and “EGCG”. For publicly available adverse event case reports, several public databases were consulted, including from the US FDA (FAERS, 2016), Health Canada (Health Canada, 2015), Australia (DAEN, 2016), and the World Health Organization (WHO, 2016). Cases were selected if MedDRA preferred terms (PT) clinically relevant to acute hepatobiliary toxicity were reported; such cases reported significant elevation in liver function tests (LFTs) or other qualifying criteria suggesting possible liver injury and/or with corroborating objective medical documentation, if available.

For the purposes of this assessment, liver toxicity was selected as the critical effect or hazard because, considering all available evidence, it is the response of human relevance that has been observed consistently across different studies and species and occurred at relatively low dose levels of EGCG with a clear dose-response; thus all publications and reports of human experience were screened to include only those that objectively or quantitatively assessed liver function and/or reported liver adverse effects. Where not reported directly, the EGCG dose used in a given study or reported in a given case report was estimated based on composition information provided in the publication or the USDA flavonoid database (Seema et al., 2014).

Results - Animal toxicity data

A total of nine publications (Bun et al., 2006; Isbrucker et al., 2006; Johnson et al., 1999; Kapetanovic et al., 2009; Takami et al., 2008; McCormick et al., 1999; Chan et al., 2010; Morita et al., 2009; NTP, 2016) covering 10 studies were identified as relevant for this analysis (i.e., included liver-related health outcomes). The test articles included brewed green tea, GTE, and purified EGCG. These were administered via oral route (both dietary feeding and gavage), and included acute, subchronic, chronic, and carcinogenicity studies. No published studies were identified testing drinking water dosing route. The weight of evidence analysis took into consideration the consistency in effects, dose-response/temporal relationship, and biological plausibility, while also taking into consideration the study quality. The purpose of this exercise was to identify overall toxicity, critical effect(s), and and associated no observed adverse effect level(s) (NOAEL). The NOAEL or lowest observed adverse effect level (LOAEL) were determined based on the adverse effects reported in the studies.

353 A NOAEL of 500 mg/kg bw/day was selected from the 13-week study in rats and pre-fed
354 dogs by Isbrucker et al. (Isbrucker et al., 2006). Application of a 100-fold safety factor derived
355 an acceptable daily intake (ADI) of 5 mg/kg/day for the EGCG preparation. Taking into account
356 that the purity of EGCG preparation was 91.8% in the study, the resulting ADI for EGCG would
357 be 4.6 mg EGCG/kg/day, equivalent to 322 mg EGCG/day for a 70 kg adult.

358 The analysis of the relevant animal studies revealed that feeding conditions are an
359 important consideration relative to an EGCG limit, as hepatotoxicity was observed at much
360 lower doses in animals exposed via oral gavage vs. dietary feeding and in the fasted vs. fed
361 states. Therefore, the above ADI is relevant for fed conditions.

362 *Results - Human intervention data*

363 From a total of 92 publications that were identified, 26 were selected, representing 27
364 studies which reported liver-related outcomes (Basu et al., 2010; Chen et al., 2016; Crew et al.,
365 2012; Dostal et al., 2015; Frank et al., 2009; Hill et al., 2007; Hsu et al., 2008; Hughes et al.,
366 2001; Joe et al., 2015; Kim et al., 2006; Lovera et al., 2015; Maki et al., 2009; Matsuyama et al.,
367 2008; McCarty et al., 2009; Mielgo-Ayuso et al., 2014; Nagao et al., 2007; Nagao et al., 2009;
368 Nguyen et al., 2012; Panza et al., 2008; Shen et al., 2010; de la Torre et al., 2016; Ullmann et al.,
369 2004; Wang et al., 2008; Wang et al., 2010; Widlansky et al., 2007; Wu et al., 2012). Test articles
370 included brewed green tea, GTE, and purified EGCG. The actual data as collected and reported
371 in different studies regarding liver function varied widely. To deal with this variability, a sliding
372 scale of relative liver function values were assigned to studies for the purpose of interpreting
373 the liver function data. A score of 1 = one report of elevated liver enzyme activity; 2.5 =

elevated mean liver enzyme activity, but still within the normal physiologic range; 10 = elevated mean liver enzyme activity above the normal range. Of the 27 studies with liver function outcomes, 20 involved patients with various disease conditions (other than liver disease). The reported or estimated EGCG dose ranged from 100 to 1600 mg/day, while duration ranged from one week to one year.

For liver function outcomes, none of the studies in healthy or diseased patients reported adverse liver effects at EGCG doses below 600 mg/day. Higher doses (>600 mg/day) were associated with a statistically significant elevation, relative to placebo or baseline levels, in liver enzyme activity within the normal range, while >800 mg/day were associated with liver enzyme activity above the normal range. From this, 600 mg/day was selected as the EGCG NOAEL, based on the combination of studies involving healthy populations (Frank et al., 2009; Hughes et al., 2002; Kim et al., 2006; Matsuyama et al., 2008; Panza et al., 2008; Ulmann et al., 2004; Wang et al., 2010). Additionally, Kim et al showed no adverse liver effects of approximately 622 mg/day EGCG in a small group of healthy smokers (Kim et al., 2006), while Ullmann et al reported one subject experienced slightly elevated liver enzyme activity at 800 mg/day (Ullmann et al., 2004).

Given the limitations of the Kim et al study (small sample size, short duration), and the absence of liver adverse effects below 600 mg/day, an uncertainty factor (UF) of 2 was selected, resulting in a proposed EGCG UL of 300 mg/day based on human intervention data.

Results - Published case and adverse event reports

A total of 22 published case reports involving green tea and liver injury were identified in PubMed (Abu el Wafa et al., 2005; Amariles et al., 2009; Bergman et al., 2009; Bonkovsky, 2006; Chen et al., 2010; Federico et al., 2007; Gallo et al., 2013; Gloro et al., 2005; Javaid et al., 2006; Jimenez-Saenz et al., 2006; Jimenez-Encarnacion et al., 2012; Lugg et al., 2015; Manso et al., 2011; Martinez-Sierra et al., 2006; Mazzanti et al., 2009; Molinari et al., 2006; Patel et al., 2013; Pillukat et al., 2014; Rohde et al., 2011; Vanstraelen et al., 2008; Verhelst et al., 2009; Yellapu et al., 2011). The post market surveillance (adverse event) data set included cases from 2006 to date (10 years) from the FDA, Health Canada, TGA, and WHO databases (FAERS, 2016; Health Canada, 2015; DAEN, 2016; WHO, 2016). Most cases were deemed unassessable due to missing or incomplete information, particularly on green tea product preparation and dose. Of those deemed causally related, no dose-response information could be derived for EGCG. Overall, the average incidence combined over the last ~10 Years was approximately 0.00364998 / 10,000, which is considered extremely rare. These findings are consistent with those presented in a recent meta-analysis by Isomura et al. (Isomura et al., 2016).

Summary of EGCG risk assessment and case/adverse event report analysis

Data source	NOAEL *	Reference	UF	UL (or ADI)	Conditions	Comments
Animal	460 mg/kg/day	Isbrucker et al 2006	100	4.6 mg/kg/day	322 mg/day in 70 kg	Proposed ADI under fed conditions

					adult	
Human	600 mg/day	Kim et al. 2006	2	300 mg/day		Proposed UL under fed conditions
Case/AE*						Incidence rate of 0.0036/10,000 -No dose response could be derived

*Based on hepatotoxicity as critical effect

Discussion

In this brief analysis, a proposed UL was identified for EGCG based on the totality of evidence from a combination of published animal toxicology, human intervention, and human case/adverse event report data. The proposed UL based on an ADI derived from animal toxicity data is 322 mg EGCG/day in a 70 kg adult. The UL based on human intervention studies is 300 mg EGCG/day in healthy adults. These values are applicable to the oral exposure under fed conditions, and consistent with those published by France (OJFR, 2014) and Italy (IMOH, 2016). The case reports and adverse event data did not provide useful information with respect to dose-response effects of EGCG. However, this information was relevant in determining an overall extremely low incidence of liver injury related to green tea, consistent with that observed with idiosyncratic hepatotoxicity (Teschke et al., 2015).

There are a number of caveats and limitations with this analysis that should be highlighted. For the human studies there were inherent limitations due to the wide heterogeneity in study design, dosage regimen, duration and population, outcome measures, and, most critically, the chemical composition of green tea preparations. For the case and adverse event reports, most could not be assessed due to missing or incomplete information, and these were not useful in yielding dose-response information related to EGCG and liver injury.

This analysis involved several assumptions, including selection of hepatotoxicity as the critical effect, a relative rating scale of liver effects between different studies, the focus on EGCG as the constituent of interest (at the exclusion of any other) and the EGCG content of preparations used in given studies, which often had to be estimated or extrapolated. Finally, it should be pointed out that the 300 mg/day EGCG proposed UL in healthy adults in a fed state may not be appropriate for traditionally prepared green tea beverages. Indeed, no UL of any kind may be necessary for this application, as there is ample evidence demonstrating health benefits above 5 cups of brewed green tea per day (Saito et al., 2015), and no documented adverse health effects in populations consuming upwards of 10 cups per day (Kazoo et al., 1997). Depending on the brewing techniques, this represents a potential EGCG exposure in excess of 1600 mg per day (Seema et al., 2014). This suggests that an EGCG UL may be best utilized to inform proper formulation of extracts or purified EGCG products.

CONCLUSION

Interest in bioactive food components continues to grow, culminating in a recent series of symposia and publications aimed at addressing whether, and if so, how, dietary guidance or recommended intakes could be established for these substances (add references). Although the aspect of safety is a critical component of any framework applied to establish intake recommendations, this has yet to be addressed for bioactives. These proceedings review the past approaches to addressing safety of nutrient substances as part of an overall framework (DRIs and essential nutrients), current approaches used by toxicologists to assess the safety of bioactives, and feature case studies as applications of these approaches. Further discussions are needed to progress the important aspect of safety of bioactives as part of an overall scientific framework that can be applied for the development of dietary guidance including recommended intakes and reference values to limit intakes where appropriate.

ACKNOWLEDGEMENTS

A conference report from the July 2016 Toxicology Forum Meeting held in Salt Lake City, UT.

We thank Dr. Jiang Hu, PhD, DABT and Dr. Kristy Appelhans, ND for their contribution and analysis that made this work possible.

CONFLICT OF INTEREST STATEMENTS

Author disclosures: Drs. Yates and Dolan have nothing to disclose. Dr. Erdman reports personal fees from Mars, Inc. and the Campbell Soup Company. Dr. Shao works for Herbalife Nutrition. Dr. Griffiths works for the Council for Responsible Nutrition (CRN). All of the authors disclosures reported above are outside the submitted work.

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