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A review on assessment of acceptable daily intake for food additives

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ABSTRACT

The acceptable daily intake (ADI) for food additives can be derived using 'no observed adverse effect level' (NOAEL) value obtained from *in-vivo* animal experiments. A safety factor (SF) of 100 is generally applied in calculation of NOAEL for the most sensitive test species to derive an ADI. The cent percent of SF is thought to be the result of individual differences in species having toxicology and toxicological dynamics. In this review, food additives are evaluated on the basis of available *in-vitro* toxicity data that could be used to derive the uncertainty factor. In addition, this paper describes the general methods adopted for assessing ADIs with the limitations inherent in these current methodologies. The methods mainly focus on a graphical display of toxicological data and estimate the acceptable intake from exposure periods for toxic substances rather than full life. Moreover, the method practices dose-response data or dose effect to calculate the decrease in the confidence level rare at the specific effect levels. These methods should lead to more inclination and be established by the Multi-type Feature Fusion (MFF) approach. It is by increasing the use of a full set of toxicological data.

Keywords: *Acceptable Daily Intake, Food Additives, EDI, NOAEL, LOAEL, FDA.*

1. INTRODUCTION

There is a clear trend for the increasing use of the *in-vitro* methods in toxicology over the last 20 years in place of animal tests [1]. Basically, the *in-vitro* studies have been adopted for hazardous characterization of several chemical substances, but they lack in considering a direct effect on the calculation of conventionally ADI values. In general, the risk of toxic chemical exposure to humans has long been evaluated using animal data, or more generally in-vivo toxicity studies. Therefore, internationally in-vivo toxicity tests and guidelines are available in the literature with two main purposes: i) to study any toxic effects of the substances on potential target tissues, and ii) to calculate NOAEL based on determination of toxic doses on the target cells or tissues.

Traditionally, the *in-vivo* animal toxicity studies which use highly sensitive animal species assess the adverse health effects to humans after taking food additives through food stuffs. In general, most of the sensitive test species have NOAEL derived from multiple doses (often determined from chronic or saprobic dietary evaluation) used to obtain ADI for a specific chemical. However, using comparable human data, dose-related toxicity on target tissue in the body [2], the identification of nearby toxins, and its response to the toxin in the cells are considered. They are highly sensitive to human and animal species combinations. To quantify the uncertainties in any of these assumptions regarding toxico-genetics and toxicodynamics or any possible mechanical differences in these individual cases, the factor of 100 (or uncertainty) is traditionally used to be highly sensitive to NOAEL in animal [3].

However, the first compound-derived toxicokinetics or Toxicodynamics data with an uncertainty factor is not considered for ADI derivation in any area of uncertainty. Hence, Renwick (1991) [4] determined a 100-fold safety factor (SF) by considering toxicokinetics (relationship between external and internal dose of a compound) and toxicodynamics (internal dose and adverse effect of a compound). Therefore, the 100-fold uncertainty factor (UF) was divided into two 10-fold SFs for the two species and for each other, respectively. Renwick (1993) [5] proposed changing this method by further dividing the security by 10 times each factor into two subfactors for toxicokinetics and toxicodynamics (TKs-TDs) which can be replaced by data obtained for the specific compound. International Program on Chemical Safety (IPCS) workshop on guidance descendants values suggested the substitution of 10 fold factor for the human variant produced by Renwick (1993) should be amended to allow for equal measuring of TKs-TDs [6]. Although the default safety factor of 100 is applied to NOAEL for derivative, the nature of the toxicity is based on toxicity data [7].

To avoid inter positive extrapolation normally the risk assessment of an external compound involves human subjects during risk trial. Although NOAEL involves human subjects (like erythrosine, stannous chloride and contraoxanthin) for ethical reasons for some dietary supplements, such studies need the known toxicology behaviors of the said test mixture. However, the mechanism of toxicity is limited to many food additives due to unknown information on impurities, so human studies become impractical [8]. At present the 100 fold uncertainty factor approach protects consumers and the safety evaluation of food additives (FAs) can be further improved by combining knowledge gained from interdisciplinary research studies. The *in-vitro* studies can consider test species under a variable human condition in decreasing gap in outcome which can be more scientific in calculating specific data derived (SF). A Joint (FAO/WHO) Expert Committee for FAs (JECFA) has reported NOAEL, lowest observed adverse effect level (LOAEL), toxicokinetics data, and *in-vitro* data at LOAEL for 65 food additive in their monograph [9]. The *in-vitro* data of 18 compounds included in JECFA monographs was absent or limited, but their inclusion helped in setting up a revised ADI (for instance by reducing a large SF). Also, the supplementary *in-vitro* and short-term *in-vivo* studies that were relevant to the main study for these compounds were collected from the scientific literature in calculating their ADI values [10]. The purpose of this review is to illustrate a revised approach to assess ADIs using toxicity data.

2. CONCEPT OF ADI

Generally, the ADI (acceptable daily intake) can be explained that a human can be exposed to a volume of a substance on a daily basis over a time period and no harmful effect was observed. It signifies negligible or no risk of harmful effects resulted in the level of daily intake of a substance in humans. It is a measure of daily exposure to the human, which is not likely a reason for harmful effects during a lifetime. ADI can be expressed in mg of the substance in consumed foods per kg of the body weight per day [11]. In order to prevent any consequence of the toxic chemical exposure, the USEPA states such an exposure quantity as a risk reference dose (RfD). Generally, RfDs are employed for health effects that are believed to have a threshold or low dose limit for generating effects [12].

The concept of ADI has later applied to establish permeable levels of contaminants in foods and beverages. The experimentally determined NOAEL was used to derive ADI when there is no statistically or biologically major symptom of the toxic effect of concern. Among different experiments with numerous NOAELs by the regulatory body the regular usage of term NOAEL is the highest experimentally established dose without a biologically or statistically significant unfavorable effect [13]. If an NOAEL could not be established experimentally then the LOAEL is applied. In standard, these safety factors (SFs) permit for inter and intra species (like animal to human) deviations with standard values of 10. To account experimental inadequacies an additional uncertainty factor can be utilized, for instance, to generalize from low exposure duration assessment to a circumstances more appropriate for chronic study or to account for insufficient numbers of test animals or other experimental confines. Conventionally a SF of 100 could be used for RfD estimation to generalize from a well accompanied animal bioassay. Such 10 fold factor is considered to accommodate animal to human and for human to human variability, 10 fold factor was also used. To adjust the uncertainty factors (UFs), if data on process modifying factors can be applied pharmacokinetics and the applicability of the animal response to human threat justify such adjustments [14]. Since, experimental data suggest that human and rat generate similar active target metabolite from a certain substance, 10 fold UFs is used to divide the NOAEL from toxicity study of animal to get a human relevant RfD, factor 3 were used for such uncertainty factor and included to make sure the protection of children and infants. For other chemical substances that have insufficient database on sub-chronic studies, factor of 10 could be judged to be more suitable for them with a SF of 1000. In sensitive humans having all required responses, the SF could be chosen for certain chemicals as small as 1, similar to the impact of fluoride in human teeth [15]. The RfD method signifies mostly an accepted method by FDA, EPA and National Academy of Sciences to establish lifetime exposure limits on humans. There are some limitations in the RfD method which include similar exposure level as RfD, but the level of hazardness would be different for different chemical substances. Also, the RfD method does not consider dose response evidence [16]. There are also complications in the implications of precise UFs within inter-species UF with the mixed results due to insufficient numbers of studied animals. Normally, the experimental results are more consistent with other studies having large numbers of experimental animals. Generally, the ADI studied by risk evaluators considers higher order of uncertainty, but the exposure level better than the ADI does not confirm the certainty level of exposure. In addition, although the ADI in which level the probability of adverse effects is less, it can't guarantee total absence of risk to people [17].

Hence, to decrease uncertainty in evaluating ADIs and RfDs, the risk evaluators are considering chemical specific adjusted risk factors instead of accepted 10 fold UFs and available data. The present WHO regulation recommends 2.5 and 4.0 fold factor for the toxicodynamics (TD) and toxico kinetic (TK) inter-species modules, whereas the inter-individual TD and TK factors of 3.16 are considered. The US regulates consumption through "disappearance data" (Food and Drug Administration, 2012*c*) where total consumption was divided by no of people consumed and 365 days for a year [18-19].

3. ESTIMATED DAILY INTAKE (EDI)

FDA assessed the estimated daily intake (EDI) of the colorants in 2010 and the EDI was around 45 mg per person per day, apart from the amounts used in cosmetics and pharmaceuticals (FDA, 2011*a*) [20]. Although the above amount was "overestimated" due to spoilage and food waste that could decrease the amounts of food consumed, FDA did not consider the skin exposure of colorants after cosmetics and creams application. Also, the people may be exposed to the additional color that can be absorbed by topical applications of Red # 33 dye available in daily cosmetic products leading to inaccurate information and doses. Actually, inaccurate information from the children (below 5 years of age) about higher consumption of snacks, chocolates, and colored cereals compare to the adult population may lead to faulty EDI value. Considering round the year social celebration children are exposed to more color combinations which warrant more exposure to dyes than most adults [21].

4. HUMAN CLINICAL STUDIES, SPECIAL ANIMAL STUDIES, OR OTHER SPECIALIZED CONSIDERATIONS BY FDA

Sometimes the standard toxicology test procedure based on animal feeding experiments of a specific additive lack to address a potential safety problem. In addition, all these animal studies don't consider related toxicity of metabolites after consumption in the testicular animal or human gastrointestinal tract. The special radio tracer based on ^{14}C radio labeled molecules may be used to assess the image of the metabolites. The issue of consistency of blood glucose levels in diabetic patients who consume a specific sugar substitute may be problematic due to an inappropriate feeding model to animals. This could be removed or minimized after feeding a pair of animals and monitoring any deficiency in calories consumed by them [22]. Nowadays petitioners are intensively designing novel food additives that can be able to convert the main caloric components of food into sugar, fiber, or fat substitutes. Since

such ingredients can be consumed in somewhat huge quantities associated with traditional food additives, the toxicity imparted by them practically might be very low. Such additives may have other potential adverse effects causing safety implications. In such circumstances, routine toxicological models cannot address the safety concerns of these additives [23].

As a result, petitioners are seeking earnestly alternative safety information which includes medical data to maintain the safety criteria of these new ingredients instead of focusing on routine toxicology studies such as (a) Reproductive effects (b) organ and cellular damage (c) gross weight enhancement or organ to body weight ratio effects. Sometimes the effects of anxiety may not be interrelated to toxicological one. Alternatively, the additive allergy issues need to be resolved before a safety decision made [24]. Under this circumstance the ability of an additive in imparting nutritional protection as well as interaction with certain drugs are important equally with their toxicological behaviour to determine their safety [25- 27]. Moreover, the petitioner has to design special methodology along with a medical evaluation to accommodate all these issues in the FDA's safety review involving the medical doctors for reviewing data collected in the medical areas. Critically, nutrition- drug correlation measurements, allergy efficacy, blood glucose homeostasis estimates, or other specialty-type studies are not suitable for a conventional EDI / ADI safety assessment comparison [28].

A multidisciplinary approach is needed to determine safety that covers many factors that are not normally considered in the traditional EDI / ADI comparison. This "multi-layered" scheme, which focuses on the weight of indication from other safety-related data's, is becoming gradually more common as part of the FDA's general rule of thumb for safety reviewers. As per FDA's regulations a new FA arrives on the market after crossing all safety issues having proofs about the integrity of the material in the context of the intended use [29].

The submitted petition would be reviewed by the FDA and the Administrative Procedures Act (APA) would scrutinize the technical note briefing the agency's conclusions on the safety of the additive before permitting the certificate for approval as a food additive in different food stuffs. Afterward the interested parties may object with supporting scientific data for consideration before the FDA [30]. The final rule as a comprehensive scientific interpretation of the agency's decision is documented as the regulation for the guidelines of the new additive in food stuffs concerned. Over the years, it has become a standard protocol for the FDA's final regulations on the use of a new additive that has a comprehensive preamble as well as any new Center for Research Code of Federal Regulations (CFR) sections that either make new use of food additives or refuse to seek use [31]. The agency's petition review details on safety assessment including the proposed use and toxicological tests conducted on the additives with a summary of the results of those experiments. The final rule acts as a legal summary to support any procedure that may be subject to procedural and judicial, if the agency's decision becomes the subject of litigation [32]. FDA compliance with this technique has given the agency to react potentially against any safety challenges related to determination procedures during the post authorization period. Throughout the history of food ingredients, there have been numerous references to the use of food products (i.e. salt, sauces, and a host of other ingredients added to the diet to perform important technical functions) and techniques (such as culture and fermentation) used in preserving and processing food [33]. To provide consumers different attractive, safe, affordable and convenient foods the FDA in partnership with the International Food Information Council developed a brochure with the following key points:

(i) the additives are added to improve or maintain safety and freshness by slowing down product spoilage caused by microbial; (ii) to improve or maintain nutritional value during processing by reinforcement and enrichment of nutritional value to reduce malnutrition in the U.S. and globally [34]; and (iii) to improve the taste, texture and appearance by adding spices, sweets and flavors enhancing the flavor of the meal.

Out of different additive colors maintain or enhance the appearance of foods, while emulsifiers, stabilizers and thickeners provide the texture and consistency of foods. Some ingredients help to control pH of the foods, while others help maintain the flavor and attractiveness of low-fat foods [35]. In addition, good manufacturing practices applicable do not exceed the required level of achievement. However, it should be safe for the ingredients in the diet for the above purposes added. It is also prohibited if the additives for this purpose do not effectively protect public health or create good public policy [36].

5. HOW DOES THE FDA DETERMINE THE SAFE LEVEL OF ADDITIVES (I.E., ACCEPTABLE DAILY INTAKE, ADI)?

ADI is the most complex measure based on data generated from animal feeding studies after scrutinization by FDA scientists. The FDA has published its recommended protocols in well-known guidelines such as the Red Book Code or the Toxicological Principles for the Evaluation of the Safety Evaluation of Food Additives. The FDA requires data to support the safety of an additive with substantial population exposure for sub-chronic toxicity tests with rodents as well as non-rodents (usually 90 days duration); reproductive studies of the additives in inducing reproductive toxic effects, or any impact on reproductive systems of an animal, or any form of birth defects; toxicity and oncology studies with rodents for 24 months in general [38]. In summary, FDA toxicologists review all data independently to detect any adverse effects on any animal size at the applied dose.

This level of exposure is often considered as the highest effect level of the admission or the HNEL. Generally, the FDA then applies 10 times to 100 times (safety fold) because the data is derived from food surveys conducted in experimental animals, not animals [39]. An additional 10 times the number of normal genetic variants are used for the vulnerable human populations and the FDA's safety factor of 100 times 'has been proven to be incredibly protective of public health. This level of intake of food additives (for instance multiplied by the NOAEL 1/100 safety factor) is considered as the ADI of the additive [40-43]. Over the years, many sophisticated techniques have been available to perform quantitative risk assessments. Proper uses of reference data in analyzing data of animal feeding experiments are important. The importance of detailed histopathology analysis is necessary to find

out correlation between HNEL and the decline in health. Actually, FDA reviewers may use one or more techniques in making food additive safety decisions, but a simple ADI / EDI comparison continues to be an adequate and effective approach [44-47].

Food additives like artificial colorants have progressively come under examination for assessment of their safety [48]. Generally, the reported quantity of artificial color used in the processed foods stuff is less than 500 $mg \cdot kg^{-1}$. Artificial colors permitted as FA in many countries have proven safe with toxicity tests. At the same time, some artificial colors have been banned due to their carcinogenicity or toxicity. The number of permitted synthetic colors varies country wise [49]. Codex Aliment Arius FAO/WHO allowed 14 artificial colors, USA 9 colors, European Union (EU) 15 colors, Korea 9 colors, Japan 12 colors and India 8 colors. Regulations like Codex Aliment Arius and EU directives control not only which synthetic colors are allowed but also monitor the permitted maximum levels. Naturally available turmeric can be used as flavoring and yellow coloring agent in foods which are manufactured from the *Curcuma* longa root with 2.5 to 6% of yellow pigments (curcuminids), dominated by curcumin. It contains curcumin (Yellow oleoresin) as a flavoring and coloring agent [50-51].

The ADI value of Curcumin was 0 - 1.0 mg/kg bw (JECFA, 1995) at a NOAEL level of 220 mg/kg bw / day in a two year dietary study in rats and SF was applied as 200. The liver was enlarged at a LOAEL of 440 mg/kg body weight/day, but hyperplasia and ulcers were developed at the level of 2000 mg/kg body weight/day in GI tract (WHO, 1995) [52]. In 2003 JECFA Curcumin set the ADI of 0 - 3.0 mg/kg body weight after studying hepatocellular changes of hepatic enzyme induction due to frequent doses of curcumin (250-320 mg/kg of body weight per day with SA of 100 [53]. Table 1 has summarized ADI values of some permitted food colour additives.

Table 1. Acceptable daily intake level of some permitted food color additives by Joint Expert Committee on Food Additives (JECFA).

S.No	Color additives	ADI ($mg.kg^{-1}$)	Reference
1	Curcumin	1.0	[2]
2	Beta-apo-8¢-Carotene	30	[54]
3	Tartrazine	7.5	[55]
4	Sunset yellow	2.5	[56]
5	Amaranth	0.5	[57]
6	Erythrosine	0.1	[58]
7	Allura Red	7.0	[59]
8	Ponceau 4R	4.0	[60]
9	Brilliant Blue	12.5	[61]
10	Indigotine	5.0	[62]
11	Fast Green	25	[63]
12	Green S	500	[64]
13	Quinoline Yellow	0.5	[65]
14	Patent Blue V	5.0	[66]
15	Na-Cu-Chlorophyllin	15	[67]
16	Annatto	0.065	[68]

6. CONCLUSIONS

The methods illustrated for calculating ADIs use most of the obtainable toxicity data and offer a reliable approach in assessing health risks for lifetime exposure of toxicants; define NOAELs considering several criticisms due to dose-response slopes and the number of animals tested. In the middle ages, these methods could be deliberated as an alternative to the current methods of establishing protection points for the toxic chemicals.

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8. ACKNOWLEDGEMENTS

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