Safety Assessment Testing Requirements for Food Color Additives

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Summary

Safety assessment of food colorants are fundamentally similar in Japan, Europe, and the United States and involve large amounts of data for new colorants and active monitoring of existing approved colorants. Testing requirement are similar for artificial and natural food colorants and include assays for genetic damage and use of animal toxicity studies as surrogates for human exposures. All studies are currently conducted in compliance with internationally recognized Good Laboratory Practices (GLP) with study design, conduct and evaluation carried out by trained and certified scientific experts in accordance with country-specific and international published safety assessment guidelines.

1. Introduction

Colorants used in foods and beverages are strictly regulated to assure consumer safety. The U.S., Europe and Japan safety assessments of food colorants are consistent with international recommendations described by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). All three countries require a large amount of data for risk assessment of food colorants with specific required information defined in guidance documents[1–8]. While safety evaluation is generally based on animal toxicity testing data, other factors that are important considerations in the safety assessment process include:

- Chemical composition of the colorant
- Presence of impurities
- Processing residues (e.g., solvents, heavy metals, etc.)
- Information on stability and degradation products
- Presence of nanoscale materials
- Justification for need of the colorant (EU)
- Demonstration of effectiveness of the colorant (EU)
- Defining the benefits of the colorant (EU)
- Steps taken to not mislead the consumer (EU)
- Manufacturing process
- Technological justification
- Allergenicity

The extent of animal toxicity testing is influenced by the estimated magnitude of human exposure and the molecular structure and properties of the colorant. It is noteworthy that the requirements for toxicity testing are fundamentally similar for natural as well as artificially produced colorants. In Europe, there is a tiered approach to safety assessment studies with a minimal requirement for toxicokinetic studies, subchronic toxicity, reproductive and developmental toxicity, and genotoxicity. Any evidence of absorption, toxicity or genotoxicity will warrant additional testing and a third tier might be required in specific circumstances. The U.S. system is basically similar with classification of the amount of safety testing dictated by levels of concern that are based on chemical structure and human exposure. The higher the level of concern, the more toxicity testing is required. In Japan, naturally derived food additives, including colorants, in use before 1995 are permitted as food colorants without limits on use level. Any colorants introduced after 1995 require a full panel of

safety assessment studies to confirm safety for the intended or applied use[6]. However, Japan's requirements for safety assessment of colorants is influenced by JECFA testing data as well as known human exposure and safety assessment data from other countries.

2. Safety Assessment Testing Guidelines

While U.S., EU and Japanese regulatory authorities may modify the extent of safety assessment testing required to safeguard consumer health, in some cases a complete battery of *in vitro* and *in vivo* animal genotoxicity tests as well as a complete series of animal studies may be required prior to permitting a new food colorant to enter the marketplace. The extent of testing required in a specific jurisdiction may be mitigated by acceptable safety assessment studies from other countries or jurisdictions. Most certainly an existing GLP-compliant study should allow for reduction of unnecessary animal testing. In contrast to safety assessment testing for drugs, colorant food additives demand a stricter safety assessment to ensure consumer safety is not compromised. This degree of vigilance is necessary as food colorants may not provide any nutritional benefit, may not provide any benefit as a food preservative or taste component, and typically are in foods consumed by all ages and throughout life. Published information on regulations and safety assessment of food colorants is available in the scientific literature[9-11].

3. Genotoxicity Assessment

The purpose of genotoxicity assessment is to determine if a food colorants can directly or indirectly change the sequence or structure of DNA in a germ cell or in a somatic cell. A change in a germ cell could result in heritable germ cell damage while a change in the DNA of a somatic cell could lead to cancer. Alternatively, a structural change in DNA could lead to cellular death. A direct effect could occur if the test material or a metabolite of the test material interacts with DNA. An indirect effect could occur if the test material altered cellular macromolecules such as mitotic spindle fibers. The types of genetic damage include mutations, chromosomal breaks, and chromosomal aberrations. The approach to genotoxicity testing might include predictive screening based on chemical structural alerts know to be detrimental, a battery of *in vitro* Good Laboratory Practice (GLP)-compliant assays, and *in vivo* GLP assays, possibly with subsequent *in vivo* GLP-compliant carcinogenicity studies. Specific written testing guidelines are available for performing genotoxicity testing and, for drugs, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) provides globally acceptable approaches for safety assessment in the U.S., Europe and Japan. ICH guidelines are applicable to safety assessment of food additives, including food colorants. In addition, the European Food Safety Authority (EFSA) provides scientific opinion on genotoxicity testing strategies applicable to food additives[12] and the U.S. Food and Drug Administration (USFDA) covers testing guidelines, including genotoxicity testing, in the FDA Redbook[1]. OECD guidelines 471, 473, 474, 475, 483, 487[7] define testing parameters for Europe. English translations of Japanese safety assessment testing guidelines are available[6,13,14].

Representative genotoxicity assays include:

- Bacterial reverse mutation (Ames) assays to detect DNA damage in bacterial organisms as a predictive screening procedure
- *In vitro* chromosomal aberration assay to detect chromosome breaks or chromosome rearrangements using cultured cells or an *in vivo* assay may be done using bone marrow cells from treated animals
- *In vitro* micronucleus assays to detect whole chromosomes or chromosome fragments that are not segregated into normal daughter cells using cultured cells, such as lymphocytes
- *In vivo* micronucleus assay to detect chromosome fragments in bone marrow and peripheral blood cells following treatment of rats or mice with the test agent
- *In vivo* Comet assay to detect DNA strand break in mammalian tissue cells such as stomach or liver using rats or mice

Transgenic rodent somatic and germ cell gene mutation

assays and other specific tests include DNA adduct measurement and assessment of DNA repair/recombination and strand breaks may be requested depending on genotoxicity test results.

4. Toxicity Assessment

In vivo animal safety assessment studies that may be required by U.S., European, and Japanese regulatory authorities are identified in published literature[9-11] and regulatory documents[1,2,4-6].

The U.S. defines minimum requirements with provision for more specific additional requirements depending on the properties of a specific food additive. The U.S. utilizes a system of classification using levels of concern that are based on the chemical structure of the additive and the anticipated human exposure (Figure 1). If a food additive is anticipated to be of low concern, then the requirements would be for a minimal amount of testing typically involving genetic toxicity tests, short term rodent toxicity studies with screening for immunotoxicity and neurotoxicity. Food additives with higher levels of concern will require more extensive toxicity testing, including rodent carcinogenicity tests as well as reproductive and developmental toxicity studies.

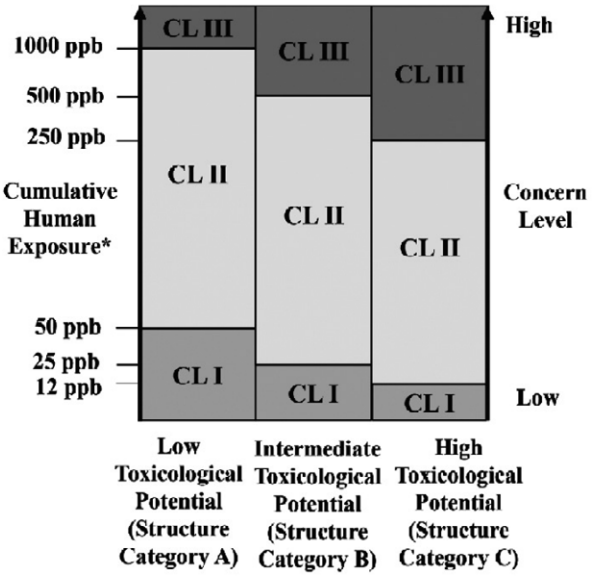

Figure 1. USFDA safety testing requirements for a specific food additive are based on levels of concern determined from the chemical structure of the additive and the anticipated human exposure.

The EU uses a tiered approach for safety testing with minimum testing including toxicokinetic studies, subchronic toxicity studies, reproductive and developmental toxicity studies, and *in vivo* genotoxicity studies. A move to a second tier would occur if there was evidence of absorption, toxicity or genotoxicity with a higher tier of requirements determine on a case-by-case basis.

Representative *in vivo* toxicity tests that may be required include:

- A 28-day rodent toxicity study as an initial safety assessment to establish testing parameters and dose levels for longer term studies. While rodent (rats and/or mice) studies are typically used to meet regulatory requirements, it is noted that current Japanese guidelines indicate possible testing in a non-rodent species in addition to a rodent study.
- A 90-day rodent toxicity study is the most common subchronic toxicity study to identify potential target organs of toxicity. It is used to identify target organ toxicity based on repeated exposure to the test agent and may provide important information for design of subsequent longer toxicity and reproductive testing parameters.
- A 1-year rodent toxicity study is the definitive long-term toxicity study to show any effects from chronic exposure to a food colorant. This study may be done as a stand-alone study or combined with a 2-year carcinogenicity study. Study design and dose levels may be based on the 90-day toxicity study and may include *in utero* exposure.
- A study to assess reproductive toxicity is typically done in rodents according to published testing guidelines. Currently an extended one-generation reproduction study is recommended and involves exposure of both male and female parents prior to mating followed by extensive evaluation of offspring.
- A developmental toxicity (teratogenicity) study is done in rodents and/or rabbits. In this study rats and/or rabbits are exposed to the test material during pregnancy and offspring are examined for developmental alterations in skeletal and soft tissues.
- A rodent carcinogenicity study is used to determine if

a test agent causes cancer. Rats and/or mice are exposed to the test material for up to two years and tissues are evaluated for evidence of preneoplastic and neoplastic lesions. This study may be combined with a one-year rodent chronic toxicity study.

- Examples of tests for allergic reactions (allergenicity) include a guinea pig maximization test and/or a local lymph node assay in mice.
- Toxicokinetics studies are important, especially for food colorants. Studies to assess test agent absorption, tissue distribution, metabolism and excretion are often carried out using radiolabeled test agent and tissue distribution may be related to target tissue pathology seen in toxicity studies.
- Behavioral and neurotoxicity studies using specific study protocols may be recommended based on clinical findings in any of the animal toxicity studies.

5. Global Safety Assessment Guidelines

The contemporary approach for determining potential hazard to humans from environmental exposures, chemical and drug exposures, and ingestion of substances added to food dates back many decades and involves testing the materials of concern in animal surrogates[15,16]. The typical studies involved exposure of laboratory rats and mice to high doses of the materials of concern followed by careful macroscopic and microscopic examinations to assess tissue toxicity. Other animal species including dogs and non-human primates may sometimes be used to carry out additional toxicity testing. Even though non-animal *in silico* testing alternatives are currently being investigated, assessment of toxicity by animal testing remains the gold standard for determining the safety of food colorants before they are permitted for human use.

Because of global marketing of food additives, the design and evaluation of appropriate safety assessments studies in experimental animals is carried out by toxicology and toxicologic pathology experts with internationally recognized credentials and specific training in physiology, pharmacology, and pathology. The requirements for training and credentials of these expert scientists is monitored by professional toxicology and pathology societies that sponsor continuing educational courses at annual meetings and by publications in scientific journals. The evaluation of toxicity study findings requires professional judgment and, since pathology diagnoses are often subjective assessments of what is or is not an adverse finding, a formal peer review procedure is typically carried out prior to submission of study results to regulatory authorities[17-20]. In an effort to globally standardize nomenclature for toxicologic pathology lesions, a series of well-illustrated publications on rodent histopathology has recently been completed by toxicologic pathologists from Europe, the U.S. and Japan[21] and current efforts are underway for similar publications for non-rodent species.

For both U.S. and EU regulatory authorities, the amount of toxicity data required for approval of a food or beverage colorant is influenced by the magnitude of human exposure and the molecular structure of the colorant. In all instances an acceptable daily intake (ADI) is calculated after applying a safety factor to a no adverse effect level (NOAEL) determined in animal toxicity studies. In contrast to regulations in other countries, naturally derived food additives, including colorants, in use in Japan before 1995 are permitted with no use level limits. For food additives introduced after 1995, the amount of toxicity data for a new food colorant required by Japanese regulatory authorities is generally greater than in the U.S. and Europe, although some consideration may be given for colorants already approved and marketed in other countries and/or supported by JECFA experts.

All national and international jurisdictions concerned with safety of food additives, including food colorants, depend on initial safety assessment studies conducted in animals prior to granting approval for use in human foods. In contrast to procedures for development of some drugs, approval of new food additives typically requires reproductive and developmental toxicity studies and carcinogenicity studies in addition to more standard toxicity testing. The animal studies must be done in compliance with Good Laboratory Practices[22].

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〔日本語訳(要旨)〕

着色料の安全性評価試験

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日本、欧州および米国における着色料の安全性評価法は基本的に類似しており、新規の着色料に関する大量のデータおよび既存の認可済み着色料の積極的な監視を必要とする。合成および天然の着色料の毒性試験要件は同様であり、遺伝毒性に係わるアッセイおよびヒトの代替として動物を用いた毒性試験が含まれる。現在、全ての試験が国際的に認められた優良試験所規範（GLP）に従って実施されており、試験のデザイン、実施および評価は、各国並びに国際的に公開されている安全性評価ガイドラインに従い、認定を受けた経験豊富な科学専門家が行っている。

PROFILE

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Dr. Hayashi received his Doctorate of Veterinary Medicine in 1985 from Osaka Prefecture University College of Veterinary Medicine, an MS in veterinary pathology from Osaka Prefecture University, completed an anatomic and molecular pathology residency at Osaka City University Medical School, and a PhD degree in veterinary and toxicologic pathology from Osaka Prefecture University. He went to National Institute of Environmental Health Sciences, North Carolina in the United States as a guest pathologist from 1999 to 2000. He is a diplomate of the Japanese Society of Toxicologic Pathologists (JSTP) as well as the Japanese College of Laboratory Animal Medicine, and is a Fellow of the International Academy of Toxicologic Pathology (IATP). Dr. Hayashi is globally recognized for his scientific expertise and serves in multiple scientific advisory roles including the IATP Accreditation Committee representative for Asia/Pacific Region, the JSTP board of Directors and Chairperson of the JSTP International Committee. He is an *ad hoc* member of Global Toxicologic Pathology President's Groups. He is also an active member of the Executive Council of Global Editorial Steering Committee (GESC) of the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND). GESC oversees the overall objectives of the INHAND projects to perform a collaborative process to review, update, and harmonize existing nomenclature documents and databases. Based on his scientific and technical credentials and scientific knowledge, Dr. Hayashi serves a current official member of the Japanese delegation for CODEX Committee of Food Additives. He serves as a council of several professional societies other than JSTP including the Japanese Society of Veterinary Science, the Japanese Society of Toxicology, and the Japanese Society of Food Chemistry. In addition to several journal editorial boards, he served the Director of Foods and Food Ingredients Journal of Japan from 2017 to 2019. He received an Outstanding Contribution in Reviewing Award from "Food and Chemical Toxicology" in 2017. He has published numerous peer-reviewed journal articles and book chapters, and most recently he co-edited the JSTP Textbook entitled "Toxicologic Histopathology" in 2017 (Nishimura Company Limited, Tokyo, Japan). He has been fostering and promoting the need for global harmonization and assessment of multiple regulatory safety requirements for food additives especially food flavorings and food colorants.

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Dr. Maronpot received his Doctorate of Veterinary Medicine in 1965 from Michigan State University, an M.S. in nutritional pathology from Michigan State University in 1966, and an M.P.H. from Harvard University in 1972. He is a Diplomate of the American College of Veterinary Pathologists as well as the American Board of Toxicology and has worked over 50 years in experimental pathology with emphasis on animal models of carcinogenesis and liver histopathology. Dr. Maronpot previously served as President of the Society of Toxicologic Pathology, President of the International Academy of Toxicologic Pathologists, served on several journal editorial boards, and was Editor-in-Chief of Toxicologic Pathology from 2001 to 2004, received the Society of Toxicologic Pathology Lifetime Achievement Award in 2008. In addition to over 300 peer-reviewed publications, he has edited a comprehensive text entitled "Pathology of the Mouse" (1999) and co-edited a book entitled "Pathology of Genetically Engineered Mice" (2000).