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GRAS determination scientific procedures and possible alternatives

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ABSTRACT

The use of a food substance is Generally Recognized as Safe (GRAS) through scientific procedures or experience based on common use in food. The pivotal data used for GRAS determination must be of common knowledge and should include evidence for safety under the conditions of intended use of the substance. Such evidence includes data on the identity and specifications of the substance, its properties of absorption, distribution, metabolism and excretion, and depending on the level of concern, data on genotoxicity, acute and subchronic toxicity, reproductive and developmental toxicity and carcinogenicity. Several alternative procedures can be used as the replacement for standard scientific procedures in order to improve the GRAS process.

1. Introduction

Prior to the introduction of any ingredient into foods, the safety of the substance is required to be evaluated under the regulations of the Food, Drug and Cosmetic Act (FDCA). One approach to safety assessment is the Generally Recognized as Safe (GRAS) determination, a science-based safety standard which requires a sponsor of a food ingredient to provide evidence demonstrating to a reasonable certainty that no harm will result from the intended use of the additive (Federal Register, 1977). According to the Food and Drug Administration (FDA) Federal Register, GRAS requires the same quantity and quality of data as a food additive petition to establish safety. Scientific procedures include human, animal, analytical, and other studies, usually published, although they can be bolstered by unpublished corroborative evidence, appropriate to establish the safety of a substance (Federal Register, 1977).

The scientific process for obtaining a GRAS determination typically begins with the Sponsor providing a dossier comprising all available data, both favorable and unfavorable, to an Expert Panel of at least 3 qualified experts from different backgrounds, who can also contribute other data to the dossier. If the data are adequate, this Panel will then approve the dossier and develop a Consensus Statement. The conclusion of this Statement is specific to the intended conditions of the use of the candidate food ingredient and represents unanimity of the Expert Panel.

2. Standard scientific procedures for GRAS determination

Preclinical technical evidence of safety as directed by the USD FDA Toxicological Principles "Redbook" (2000) involves providing data on the identity and specifications of the additive, its properties of absorption, distribution, metabolism and excretion. Also required is documentation of genotoxicity testing, acute toxicity testing, results of a 90-day study for subchronic toxicity, reproductive and developmental toxicity and carcinogenicity testing, depending on the level of concern. A systematic approach, the Decision Point Approach (Fig. 1), helps to select the battery of tests to evaluate the genotoxic and carcinogenic potential of substances at the earliest stages. The substance itself must be well characterized, and the substance used in toxicology testing must be identical to the product for GRAS determination. This includes plant extracts and mixtures.

Substance identity and specifications include the structure category assignment developed by FDA, which is based on the information on the toxicological potential of the substance predicted from its chemical structure. The assignment is divided into 3

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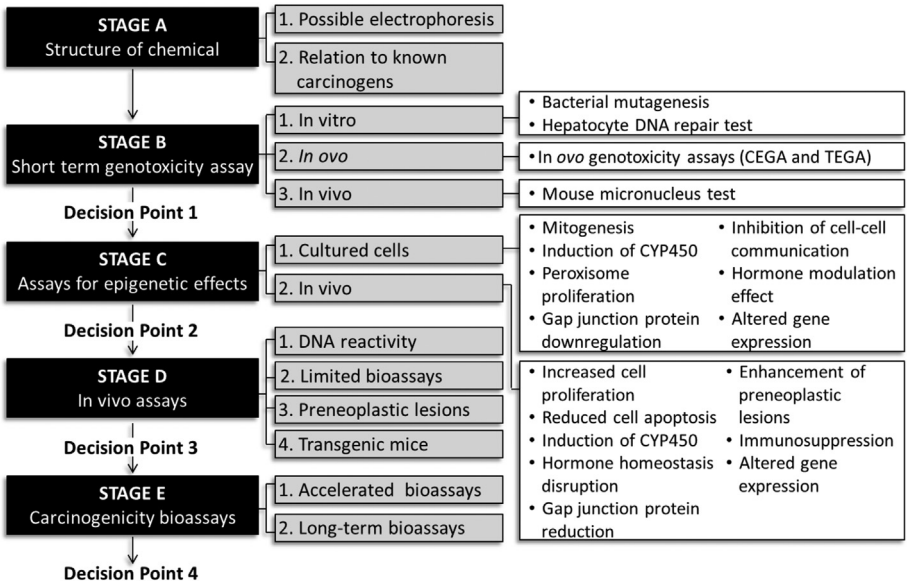

Fig. 1. Decision point approach in carcinogen testing. Decision Point 1, evaluation of findings in stages A and B; Decision Point 2, evaluation of results from stages A through C; Decision Point 3, evaluation of results from stages A to C and selected tests in stage D; Decision Point 4, final evaluation of all results and cancer hazard assessment. Modified from Hayes' Principles and Methods of Toxicology, 6th ed., 2014 (Williams et al., 2014b).

categories: A, for low toxic potential; B, where there are adverse effects other than mutagenicity and carcinogenicity; and C, where the substance is structurally related to reported mutagens or carcinogens. Another tool used by FDA at this point for the assessment of the probability of human adverse effects from low levels of exposure is a Threshold of Toxicological Concern (TTC) approach. Data for this assessment is usually collected from animal studies. The main goal of deriving a TTC is to identify the level of exposure to a chemical below which there would be no adverse health effects. A structural category assessment scheme (tree) created by Cramer et al. (1978) is widely used in TTC estimation. This tool uses data on the chemical structure, recognized routes of metabolism, toxicity data and measurements of total human intake of a compound. The classification includes 3 classes: Class I, substances with simple chemical structure and known metabolic pathways which suggest a low order of metabolic toxicity; Class II, substances that are intermediate; Class III, substances with chemical structures which may suggest a significant toxicity. The structural category assessment contributes to the determination of priorities for testing. At this level, computational toxicology could be also involved.

In order to determine which toxicity tests are needed to assess safety, FDA also assigns compounds to Concern Levels that are similarly divided into three categories; Concern Level I or low; Concern Level II or intermediate; Concern Level III or high. These levels are determined by several considerations, including potential cumulative human exposure and the structure category assignment as described above. Usually, information regarding exposure has more weight than structure alert information. Compounds that belong to Concern Level I require only genotoxicity and acute toxicity testing; those that belong to Level II additionally need data from subchronic and reproductive and developmental toxicity studies; and finally, if a compound is assigned to Concern Level III, one year non rodent and carcinogenicity studies should be also provided.

The Absorption, Distribution, Metabolism, Excretion (ADME) studies must be done for the species to be used for the toxicity studies, and in the results, the animal pharmacokinetics must replicate possible pharmacokinetics in humans.

Acute toxicity results are obtained by single or repeated dosing in rats or mice and observing them for 14 days. At termination, necropsy and histopathology results are evaluated. Experimental values involve the median lethal dose (LD_{50}) and the test subjects are observed for any adverse effects level to determine no-observed-adverse-effect-level (NOAEL) and low-observed-adverse-effect-level (LOAEL).

Subchronic toxicity testing involves a 90-day study in rats or mice and is justified based on ADME. Rodents are exposed to four different levels of a test article (3 doses and control). Standard parameters for results include comprehensive histopathology. Other parameters include genetic toxicology, a functional observational battery (FOB), and bioindicators of effect, e.g. gene expression data.

Reproductive and developmental toxicity data becomes necessary if there is any indication of reproductive organ toxicity in the findings from the acute or sub-chronic studies that have been conducted. Pharmacokinetic and metabolic data are used to select the most appropriate species for this testing, usually rats or rabbits. If neither is applicable, then the most sensitive species should be used, since according to FDA, humans are more sensitive to reproductive toxicity than any animal model.

Genetic toxicology utilizes a battery of in vitro and in vivo assays. In vitro studies involve a bacterial mutagenicity (Ames) assay and testing for gene mutation in mammalian cells, usually mouse lymphoma cells using thymidine kinase locus mutation. In vivo tests include cytogenetic damage, and induction of mouse bone marrow micronuclei. However, the latter assay has limitations as there can be false positive results (resulting from induction of hematopoiesis) and false negative results (where the chemical is not metabolized in the bone marrow or the active metabolite does not reach the bone marrow). The data obtained from an extensive study in mice provided by Morita et al. (1997) on the evaluation of the micronucleus assay in the screening of the human carcinogens determined by International Agency for Research on Cancer (IARC) reflects this situation. Thus, only 68.6, 54.5 and 45.6% of the carcinogens belonging to IARC Groups 1, 2A and 2B respectively,