Scientific procedures and possible alternative assays in GRAS determination

A food substance can be designated as Generally Recognized as Safe (GRAS) based on the outcomes of scientific procedures or experience of common use in food. Data used for GRAS determination is provided by the Sponsor and is derived from human, animal, analytical, and other studies, usually published, although they can be supported by unpublished corroborative evidence, appropriate to establish the safety of a substance under the conditions of intended use (Federal Register 1977). 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 for safety, data on genotoxicity, acute and subchronic toxicity, reproductive and developmental toxicity and carcinogenicity (Tab. 1).

To decide which toxicity assays are needed for safety assessment, the Food and Drug Administration (FDA) assigns compounds to Concern Levels: Concern Level I, or low, requires only genotoxicity and acute toxicity testing; Concern Level II, or intermediate, additionally needs data from subchronic, reproductive and developmental toxicity studies; for Concern Level III, or high, one year non rodent and rodent carcinogenicity studies are necessary (Tab. 1). This classification is determined by several considerations, including structure category assignment and potential cumulative human exposure. Based on the chemical structure a compound can be assigned one of the following categories: A, low toxic potential; B, adverse effects other than mutagenicity and carcinogenicity; C, structurally related to reported mutagens or carcinogens. At this level, computational toxicology could be also involved.

Clinical technical evidence of safety involves human studies. Also data in the Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS), which requires reporting of serious adverse events for dietary supplements, is relevant.

Based on the outcomes of available studies, an independent Expert Panel of at least 3 qualified experts from different backgrounds, must unanimously conclude that other qualified scientists would agree with the conclusion (i.e., there would be a “consensus” that the substance is GRAS). This conclusion is specific to the intended conditions of the use of the candidate food ingredient.

The use of alternative procedures can significantly improve the GRAS process (Williams *et al.*, 2014[a]; 2014[b]). Among such alternatives are *in Ovo* Genotoxicity Assays, i.e. Chicken Egg Genotoxicity Assay (CEGA) and Turkey Egg Genotoxicity Assay (TEGA), that have been developed as an enhanced tool for the assessment of the potential of a chemical to induce DNA damage (Kobets *et* al., 2016; Williams *et al.*, 2014 [a]). The model, which is intermediate between in vitro and in vivo assays, uses fertilized avian eggs injected with the test compound for 3 days. The endpoints of the assays include detection of DNA adducts and DNA strand breaks, evaluation of the histopathological changes, and for the chicken, analysis of gene expression profile. The model

has multiple advantages, and potentially can substitute for in vivo testing.

Study	Description	Concern Level
Absorption, Distribution, Metabolism, Excretion (ADME)	• Conducted for the species to be used for the toxicity studies • The animal pharmacokinetics must replicate possible pharmacokinetics in humans	I
Acute toxicity	• 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}), no-observed-adverse-effect-level (NOAEL) and low-observed-adverse-effect-level (LOAEL).	I
Subchronic toxicity	• 90-day study in rats or mice. • 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.	I, II
Reproductive and developmental toxicity	• Becomes necessary if there is any indication of reproductive organ toxicity in the findings from the acute or sub-chronic studies. • 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.	I, II
Genetic toxicology	• 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.	I, II, III
Carcinogenicity bioassays	• Are recommended in two rodent species dosed with four doses (3 doses and control) for 2 years with in-life observation. • The results of the bioassay include necropsy, clinical pathology, and comprehensive histopathology performed on at least 40 organs or tissues. • A panel of qualified pathologists (Pathology Working Group) carefully examines any lesion identified by the primary pathologist. • This testing is not indicated for naturally occurring substances which are components of food (e.g. amino acids).	I, II, III

Tab. 1. Scientific procedures involved in GRAS determination.

The Accelerated Carcinogenicity Bioassay (ACB), designed as an alternative to the carcinogenicity testing (Iatropoulos *et al.,* 2001; Williams *et al.,* 2014[b]), is conducted in either rats and/or mice and involves ancillary endpoints (e.g. cell proliferation, preneoplastic lesions). The endpoints are assessed in six or more critical target tissues, after administration of test substance for 16 weeks as possible cancer initiator followed for 24 weeks either by controlled diet or a known tumor promoter; or the administration of the test substance for 24 weeks as a possible promoter after administration of known initiating carcinogen for 16 weeks.

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