National Standards of People's Republic of China

GB 15193.1-2014

National Food Safety Standard

Procedures for Toxicological Assessment of Food

Issued on: 2014-12-24 Implemented on: 2015-05-01

Issued by National Health and Family Planning Commission

National Standard for Food Safety

Procedures for Toxicological Assessment of Food

1. Scope

This Standard specifies the procedure for toxicological assessment of food.

This Standard is applicable to the assessment of the safety of chemical, biological and physical factors that may cause harm to health concerned during food production, processing, storing, transport and selling process. Assessment objects include food and its raw material, food additives, new food raw materials, irradiated food, food related products (used for food packaging materials, containers, detergent, disinfectant; and the tools and equipment used for food production and marketing), and food pollutants.

2. Requirements for test substances

2.1 It shall provide the test substance's name, batch number, content, storage conditions, and sources of raw materials, production processes, quality specifications, character, human recommended (possible) intake, and other relevant information.

2.2 For single-ingredient chemical substance, the physical, chemical property (including chemical structure, purity, stability, etc.) of test substance (including impurities when necessary) shall be provided. For mixed substances (including formulated products), the composition of test substance shall be provided; when necessary, physical property, chemical property (including chemical name, structure, purity, stability, solubility, etc.) of all compositions of test substances and relevant data shall be provided.

2.3 If the test substance is formulated product, it shall be the standardized product; its composition ingredients, proportion and purity shall be the same as the actual application. If the test substance is an enzyme preparation, it shall, before other compound ingredients are added, use the product as the test substance.

3. Contents of toxicological assessment for food safety

3.1 Acute oral toxicity test

3.2 Genetic toxicity test

3.2.1 Genetic toxicity test contents. Bacterial reverse mutation test, mammalian erythrocyte micronucleus test, mammalian bone marrow chromosome aberration test, mouse spermatogonia or spermatocytes chromosome aberration test, in-vitro mammalian cell HGPRT gene mutation test, in-vitro mammalian cell TK gene mutation test, in-vitro carcinogenicity studies (or chronic toxicity and carcinogenicity merge test).

3.2.2 Genetic toxicity test combinations. Generally speaking, these combinations shall comply with the principle of integrating prokaryotic cells and eukaryotic cells together and integrating in-vitro tests and in-vivo tests together. The following combinations are recommended in accordance with properties of the test material and the test purpose:

Combination 1: Bacterial Reverse Mutation Assay: Mammalian Erythocyte Micronucleus Test or Mammalian Bone Marrow Cell Chromosome Aberration Test, Mouse Spermatogonial / Spermatocyte Chromosome Aberration Test or Rodent Dominant Lethal Test.

Combination 2:Bacterial Reverse Mutation Assay: Mammalian Erythocyte Micronucleus Test or Mammalian

Bone Marrow Cell Chromosome Aberration Test, In Vitro Mammalian Cells Chromosome Aberration Test or In Vitro Mammalian Cell TK Gene Mutation Test.

Other Optional Genotoxicity Tests: Sex-linked Recessive Lethal Test in Drosophila melanogaster, In-vitro Mammalian Cells Repair of DNA Damage (Unscheduled DNA Synthesis (UDS)) Tes, In-vitro Mammalian Cells HGPRT Gene Mutation Test.

3.3 28-day Oral Toxicity Test

3.4 90-day Oral Toxicity Test

3.5 Teratogenicity Test

3.6 Reproductive Toxicity Test and Reproductive & Developmental Toxicity.

3.7 Toxic kinetic Test

3.8 Chronic Toxicity Test

3.9 Carcinogenesis Test

3.10 Chronic Toxicity and Carcinogenicity Test

4. Principle for Selection of Toxicity Tests

4.1 For substances initiated in China, especially those substances whose chemical structures indicate potential chronic toxicity, genotoxicity or carcinogenicity, or those substances of large output, wide range of application and large human intake, systematical toxicity tests will be required, including acute oral toxicity test, genotoxicity test, 90-day oral toxicity test, malformation test, reproductive & developmental toxicity test, toxicokinetic test, chronic toxicity test and carcinogenesis test (or chronic toxicity and carcinogencity test).

4.2 For derivatives or analogs of which the chemical structure is basically same as that of known substances (refer to those that have passed safety evaluation and are allowed for use), or the substances that have safe-use history in some countries and regions, then it may firstly conduct the acute oral toxicity studies, genetic toxicity test, 90-day oral toxicity test, and teratogenicity test. According to the test results, determine whether it needs to conduct the toxicokinetic test, reproductive toxicity test, chronic toxicity test and carcinogenicity test, etc.

4.3 For substances that are known or have use history in several countries, at the same time, the application organization has the data to prove that the quality specifications of the declared test substance are consistent with foreign products, then it may firstly conduct the acute oral toxicity test, genetic toxicity test, and 28-day oral toxicity test. According to the test results, determine whether it needs to further conduct toxicity test.

4.4 Selection of the safety toxicology assessment test for food additives, new resources of food and ingredients, food related products, pesticide residue, veterinary drug residue

4.4.1 Food additives

4.4.1.1 Flavoring

4.4.1.1.1 All the flavorings that have been approved for use or have been formulated for daily acceptable

intake by World Health Organization (WHO), and allowed by two or more organizations of WHO, FEMA, COE and IOFI, then it generally is not required to conduct test.

4.4.1.1.2 If the data is incomplete or is only approved by one of the international organizations, then acute toxicity test and one of genetic toxicity test combination shall be conducted first; decide whether further test is needed after preliminary assessment.

4.4.1.1.3 If no data can be referred to and it is not yet allowed to use by international organization, then conduct the acute toxicity test first, genetic toxicity test and 28-day oral toxicity test. Decide whether further test is needed after preliminary assessment.

4.4.1.1.4 For single high-purity natural flavoring that is extracted from edible part of animal or plant, if the chemical structure and relevant data do not show un-safety, then toxicity test is generally not required.

4.4.1.2 Enzyme preparations

4.4.1.2.1 For enzyme preparations that have long history of safe consumption and that are produced from edible parts of animals or plants, if the World Health Organization has announced the acceptable daily intake (ADI) or ADI is not required to be specified or several countries have approved the use, on the basis of providing relevant proving.

4.4.1.2.2 For enzyme preparations obtained from other sources: if relatively complete toxicology data is available, and WHO has defined the acceptable daily intake or it is not necessary to define the acceptable daily intake, or the preparation has been approved for use in several countries，it is required to carry out the acute oral toxicity test and the genotoxicity test in the case the preparation's quality complies with corresponding international quality standards, and it is required to carry out the acute oral toxicity test, the genotoxicity test, and the 28-day oral toxicity test in the case the preparation's quality does not comply with corresponding international quality standards，and the question whether to execute other toxicity tests depends on the results of the tests required.

4.4.1.2.3 For enzyme preparations obtained from other sources: if the preparation is a new species, it is required to carry out the acute oral toxicity test, the genotoxicity test,the 90-day oral toxicity test and the malformation test first for preliminary assessment，and whether to carry out further tests depends on the results of the preliminary assessment; if the preparation has been approved for use in one country and the WHO has not defined the acceptable daily intake or no complete data available, it is required to carry out the acute oral toxicity test, the genotoxicity test, the 28-day oral toxicity test，and whether to carry out further tests depends on the results of the tests required.

4.4.1.2.4 Testing on enzyme preparations produced by transgene should comply with applicable regulations on management of transgenosis of the state.

4.4.1.3 Other Food Additives

4.4.1.3.1 If relatively complete toxicology data is available, and WHO has defined the acceptable daily intake or it is not necessary to define their acceptable daily intake, or the additive has been approved for use in several countries，it is required to carry out the acute oral toxicity test and the genotoxicity test in the case the additives' quality complies with corresponding international quality standards, and it is required to carry out the acute oral toxicity test, the genotoxicity test, and the 28-day oral toxicity test in the case the additives' quality dose not comply with corresponding international quality standards，and the question whether to execute other toxicity tests depends on the results of the tests required.

4.4.1.3.2 If the additives have been approved for use in one country and the WHO has not defined the acceptable daily intake or no complete data available, it is required to carry out the acute oral toxicity test, the genotoxicity test, the 28-day oral toxicity test，and the teratogenicity test, and whether to carry out further tests depends on the results of the tests required.

4.4.1.3.3 For single components prepared from animals, plants, and microorganisms, and high purity food additives, if the component/additive is a new species, it is required to carry out the acute oral toxicity test, the genotoxicity test, the 90-day oral toxicity test and the teratogenicity test first for preliminary assessment, and whether to carry out further tests depends on the results of the preliminary assessment; if the component/additive has been approved for use by an international organization or in one country it is required to carry out the acute oral toxicity test, the genotoxicity test and the 28-day oral toxicity test，and whether to carry out further tests depends on the results of the tests required

4.4.2 New food raw materials

It shall be evaluated in accordance with "New food raw material declaration and acceptance provisions" (State-Health-Food-Announcement [2013] 23).

4.4.3 Food related products

It shall be evaluated in accordance with "Food related product new varieties declaration and acceptance provisions" (Health-Supervision-Announcement [2011] 49).

4.4.4 Pesticide residue

It shall be evaluated in accordance with GB15670.

4.4.5 Veterinary drug residue

It shall be evaluated in accordance with "Veterinary drug before-clinical toxicology evaluation test guidelines" (The People's Republic of China, Ministry of Agriculture Bulletin No. 1247).

5. Objective of toxicological assessment of food and result judgment

5.1 Objectives of toxicological test

5.1.1 Acute toxicity test

Understand the degree of toxicity, property and possible target organ of test substance; provide basis for selection of dose and toxicity observation index for further toxicity test; classify the acute toxicity dosage according to LD50.

5.1.2 Genetic toxicity test

Screen the genetic toxicity of test substance and whether it has potential carcinogenesis and cell mutagenicity.

5.1.3 28-day oral toxicity test

On the basis of the acute toxicity test, understand more about the nature of the test substance’s toxicity, the dose-response relationship and possible target organs, so as to obtain the 28-day oral not-observed adverse effect dose; preliminarily evaluate the safety of the test substance; and provide the basis for

selecting longer-term toxicity and chronic toxicity test dose, observation indicators, toxicity endpoint for next step.

5.1.4 90-day oral toxicity test

Alternative tests (at least one is in-vivo test). If the additional 2 alternative tests are negative, it may proceed to the next toxicity test; if 1 item is positive, it shall give up that the test substance can be used in food.

5.1.5 Teratogenicity Test

The test is to determine whether a test substance has teratogenicity and development toxicity，and define the test substance's NOAEL(No Observed Adverse Effect Level) for teratogenicity and development toxicity.

5.1.6 Reproductive Toxity Test and Reproductive & Developmental Toxicity

The test is to understand how a test substance toxic affects the laboratory animal breeding and its offspring growth, in terms of gonadal function, estrous cycle, mating behaviour, pregnancy, childbirth, breastfeeding and weaning, and its offspring reproductive and development. After obtaining the unobserved harmful effects dose levels by the test substances, it provides the scientific evidence of developing the preliminary population safe lifting limits standard.

5.1.7 Toxicokinetics Test

The test is to explain how a test substance is absorbed and distributed in vivo and how soon will be excreted and other related information, to provide basis for selection of suitable germline of experimental animals used in the chronic toxicity test, and to reveal the formation of metabolites.

5.1.8 Chronic Toxicity Test and Carcinogenesis Test

The test is to explain a test substance's toxicity and carcinogenicity of long-term exposure, to define the test substance's NOAEL, and to provide basis for the final assessment for application in foods and the determination of HBGV (health-based guidance values).

5.2 Analysis on Results of Various Toxicology Tests

5.2.1 Acute Toxicity Test

Generally speaking, if a test substance's LD50 (Lethal Dose, 50%） is less than 100 times of the human recommended (possible) intake, the substance should not be used in food, and no further toxicology tests is necessary.

5.2.2 Genotoxicity Tests

5.2.2.1 It is very likely that the test substance has genotoxicity and carcinogenicity if the results of two or more tests in the genotoxicity test combination are positivethe, and the substance should not be used in food if so.

5.2.2.2 If the result of one test in the genotoxicity test combination is positivethe，two more optional tests（one in vivo test at least）shall be selected and carried out. If the results of the tow optional tests are both positive, further toxicity tests shall be carried out；if the result of one optional test is positive, the test substance should not be used in food.

5.2.2.3 If the results of the three tests are positive, further toxicity tests shall be carried out.

5.2.3 28-day Oral Toxicity Test

For test substances only the acute toxicity test, genotoxicity test and 28-day oral toxicity test are required to be carried out, a preliminary assessment could be obtained combining with results of other tests, if no evident toxicity is observed in the acute toxicity test, genotoxicity test and 28-day oral toxicity test; and further toxicity tests should be carried out if evident toxicity is observed, especially a dose-response relationship exists.

5.2.4 90-day oral toxicity test

According to the not-observed adverse effect dose obtained by the test to conduct assessment, the principle is:

a) If not-observed adverse effect dose is less than or equal to 100 times of recommended (possible) human-intake, it indicates stronger toxicity; it shall give up that the test substance can be used in food;

b) If not-observed adverse effect dose is more than 100 times but less than 300 times, it shall conduct chronic toxicity test;

c) If not-observed adverse effect dose is more than or equal to 300 times, then chronic toxicity test is not required; it may proceed to safety assessment.

5.2.5 Teratogenicity Test

According to the test results, evaluate if the test substance is the teratogenic substance to laboratory animals. If teratogenic test result is positive, then reproductive toxicity and reproductive-developmental toxicity test shall not be continued. For other developmental toxicities that are observed in teratogenicity test, it shall combine with 28-day and (or) 90-day oral toxicity test results to conduct the assessment.

5.2.6 Reproductive toxicity test and reproductive-developmental toxicity test

According to the not-observed adverse effect dose obtained by the test to conduct assessment, the principle is:

a) If the NOAEL is lower than or equal to 100 times of the human recommended (possible) intake, the test substance should not be used in foods.

b) If the NOAEL is higher than 100 times but lower than 300 times of the human recommended (possible) intake, the chronic toxicity test should be carried out.

c) If the NOAEL is higher than 100 times or equal to 300 times of the human recommended (possible) intake, a safety assessment could be obtained without carrying out the chronic toxicity test.

5.2.7 Chronic Toxicity Test and Carcinogenesis Test

5.2.7.1 The principle for assessment according to the NOAEL obtained in the chronic toxicity test is as follows:

a) If the NOAEL is lower than or equal to 50 times of the human recommended (possible) intake, the

test substance could have strong toxicity and should not be used in foods.

b) If the NOAEL is higher than 50 times but lower than 100 times of the human recommended (possible) intake，the question whether the test substance could be used in foods depends on the safety assessment.

c) If the NOAEL is higher than 100 times of the human recommended (possible) intake, the test substance allowed to be used in foods.

5.2.7.2 The principle to analyse the result of the carcinogenesis test according to the incidence, incubation period and multiplicity determined in the carcinogenesis test is as follows（If one or more of the following conditions are met， the result of the carcinogenesis test could be regarded as positive; if a dose-response relationship exists, it could be more certain about the positive result.）:

a) Cancer only occurs among animals of the test group.

b) Cancer occurs among animals both of the test group and the control group, but the incidence of the test group is higher.

c) The multiplicity of cancer is evident in the test group, while no multiplicity exists or only seldom animals have gotten multiple cancer in the control group.

d) There is no evident difference in the incidence between the test group and the control group, but cancer occurred earlier in the test group.

5.2.8 Others

If the highest dosage of the test substance used as feed additive(no more than 10% of the feed, in principle) or the dosage after concentration (liquid test substances)is lower than the required times of the human recommended (possible) intake specified according to the NOAEL，a safety assessment could be made combining results of other toxicity tests and the actual intake.

6. Factors to Consider During Food Safety Assessment

6.1 Test indicators' statistical significance, biological significance and toxicological significance

For the abnormal changes of some indicators in experiment, it shall, according to if there are statistical differences between experimental-group and control-group's indicators; if there is dose-response relationship; the horizontal comparison of similar indicators; and consistency of both sexes and of the historic control value range of that laboratory; etc., comprehensively consider if the indicator difference has biological significance. And further judge if there is toxicological significance. In addition, if some tumor is found in experimental-group but not in control-group, even it has no statistical significance with the control-group, it still needs to be paid attention to.

6.2 Test substance of larger recommended (possible) human-intake

It shall consider that, when the given test substance is too high, it may affect nutrient intake and bioavailability, so as to cause some toxicology performance, rather than that the toxicity is caused by the test substance.

6.3 Time-toxic effect relationship

When conducting analysis and assessment to toxic effects of experimental animals that are caused by the test substance, it shall consider that, at the same dose level, the toxic effects change along the time.

6.4 Special human-groups and vulnerable human-groups

For the foods eaten by pregnant women, nursing mothers or children, it shall specially pay attention to its embryo toxicity or reproductive-developmental toxicity, neurotoxicity and immunotoxicity etc.

6.5 Human-group information

Due to the species difference between human being and animal, while assessing the food safety, response data of human being after contacting test substances shall be collected as much as possible, such as occupational contact and accidental contact, etc. Under the condition of ensuring safety, human tasting test can be considered in accordance with relevant regulations. And volunteer testees' toxicokinetics or metabolism data is of important significance to deduct the animal test result to human being.

6.6 Animal toxicity test and in-vitro test data

The various animal toxicity tests and in-vitro test systems listed in this Standard are the most important data that can be obtained under the current management (regulations) toxicological assessment level; it is also the main basis for safety assessment. When test result is positive, and the result judgment is involved in whether the test substance could be applied in food, the repeatability of result and dose-response relationship shall be considered.

6.7 Uncertainty coefficient

It is the safety coefficient. When deducting the animal toxicity test result to human being, because there are biological differences among animal, human being, and human individual, uncertainty coefficient is usually 100; however, it may comprehensively consider the number of safety coefficient according to the test substance's raw material source, physiochemical property, degree of toxicity, metabolism characteristic, accumulation, contacted human-group scope, usage amount in food and possible intake for human being, application scope and function, and other factors.

6.8 Data of toxicokinetics test

Toxicokinetic test is an important aspect for toxicological assessment to chemical substances, because different chemical substances or dose amount often have significant impact to the differences of toxicokinetic or metabolism. In toxicity test, the animal species with the same metabolism method and mode as human being shall be, in principle, applied as much as possible for test. Studying the difference of test substance's absorption, distribution, excretion and biotransformation on animal and human being has important significance on deducting the animal test results to human being and reducing the uncertainty.

6.9 Comprehensive assessment

While conducting final assessment, the physiochemical property, structure, degree of toxicity, metabolism characteristic, accumulation, contacted human being scope, usage amount and usage scope in food, recommended (possible) intake for human being, and other factors of test substance shall be comprehensively considered; For the substances that have been applied in food for a relative long-time, they are of great significance to the epidemiology investigation for contact people; however, it is usually difficult to obtain the reliable data on dose-response relationship. For new test substances, only animal test

and other test study data can be relied on. However, even if there are complete and detailed animal test data and some human being contact epidemiology study data, it is still hard to make assessment that it could guarantee every person's safety due to the different species of human being and individuals. The so-called absolute safety actually does not exist. It shall comprehensively balance between that it may possibly cause harm to human-body health and it may provide benefits; on premise of food safety, the basis of safety assessment is not only the result of safety toxicology test, but also relates to the then-level of science, technological conditions, socio-economy, and cultural factors. Thus, along the time, the development of social economy, scientific and technological progress, it is necessary to re-assess the test substances that have previously passed the assessment.