

## Food additives

### *Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives*

Geneva, December 2000

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## PREFACE

This edition of the guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives (JECFA) replaces the guidelines issued in 1989 (ICS/89.40).

These guidelines are intended primarily for WHO Temporary Advisers who prepare working papers for JECFA and for Members who have been assigned to peer review them and propose evaluations. The guidelines are also useful to manufacturers who wish to prepare summaries of data that they provide. The WHO Secretariat encourages the submission of such summaries by manufacturers.

Major changes to the previous edition of the guidelines are the following:

1. Modification of headings for toxicological studies. It should be noted in particular that multigeneration reproductive toxicity and developmental toxicity studies should be grouped under **reproductive toxicity**.
2. A description of the procedure for the preparation of consolidated working papers by Temporary Advisers and Members is provided (section 1, paragraph 2).
3. Temporary Advisers are requested to prepare a *table of contents* for each working paper (section 2.2).
4. Temporary Advisers are requested to include biochemical transformation schemes, if appropriate for the chemical (section 2.4.1.2)
5. A sample report item has been included as an appendix, which should serve as a model for the preparation of the **Comments** and **Evaluation** sections (Appendix E).
6. Expanded and revised guidance on organization and punctuation of references for citation in the text and listing at the end of the working paper (section 2.8).
7. Separate guidelines have been developed for the working paper (monograph) format for flavouring agents. These guidelines are available upon request from the WHO Joint Secretary of JECFA.

These guidelines are available from the WHO Joint Secretary, Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, 1211 Geneva 27, Switzerland. Comments on these guidelines and suggestions for future editions are gladly accepted.

# GUIDELINES FOR THE PREPARATION OF TOXICOLOGICAL WORKING PAPERS ON FOOD ADDITIVES FOR THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

## 1. Introduction

These notes are designed to guide WHO Temporary Advisers in the preparation of toxicological working papers for consideration at meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). These working papers summarize the toxicological and related data that are used for making quantitative determinations of safety of food additives. When possible, acceptable daily intakes (ADIs) are established on the basis of these data.

Temporary Advisers are requested to prepare working papers using these guidelines. Their working papers should include all relevant sections except for the **Evaluation** section. The draft working paper is then transmitted to the Member who has been assigned the compound for comments and a proposed evaluation. The Temporary Adviser and Member should work together to prepare a consolidated working paper that contains all relevant sections, which is submitted to the WHO Secretariat in sufficient time before the meeting for reproduction and distribution to other Members.

Most working papers are published after meetings of the Expert Committee as toxicological monographs in the **WHO Food Additives Series**. To facilitate their editing and to avoid delays in their publication, the WHO Secretariat would appreciate close adherence by Temporary Advisers to the standard style described in these guidelines.

The working paper pattern is outlined in Appendix A, which shows the order in which items appear and numbering style. Boldfacing should be used with all titles and headings.

Working papers should be submitted in **single spacing**. They should be provided in electronic format on diskette at the time of the meeting. If a Macintosh computer is used, the file should be converted to PC format before submitting it to the Secretariat.

Two types of toxicological monographs are published after the meeting, full monographs and monograph addenda. Full monographs are published on food additives that are reviewed by the Committee for the first time and on which comprehensive re-evaluations are performed. When limited data are evaluated, monograph addenda are usually prepared, which summarize the relevant safety data that have become available since the most recent evaluation; they do not contain the summaries included in earlier monographs. The same pattern is followed with both, although addenda usually contain fewer sections than full monographs.

## 2. Working paper structure and content

This section summarizes the types of information that are usually included in toxicological working papers and the way that this information should be organized. The studies that are listed do not comprise a checklist of required studies. Rather, they are included to provide guidance on ways the usual types of data should be summarized and organized (see Appendix A).

**2.1 Title** - The main title should be the name usually given to the food additive.

**2.2 Table of contents** - A table of contents consisting of section headings and page numbers should be included on the first page of the working paper (for an example see a recently published monograph in the WHO Food Additives Series).

**2.3 Explanation** - If the food additive has not been evaluated previously by the Expert Committee, it should be so stated, along with a brief description of the substance and its primary uses. If it has been evaluated before, previous evaluations should be referenced by number using the standardized reference list of JECFA publications, which may be found in annexes to recent JECFA reports (WHO Technical Report Series) and evaluations (WHO Food Additives Series). Thus, the report of the forty-ninth meeting would be referenced as (Annex 1, reference 131) and a toxicological monograph prepared after the forty-ninth meeting would be referenced as (Annex 1, reference 132). Reference to previous Committees should be made by number (such as the forty-sixth meeting of the Committee), rather than by year, because in some cases reports have not been published in the same year as the meeting and in many years two meetings have been held, which would create confusion if meetings were referenced by year. Reasons for the present re-evaluation should be given and, if a full monograph on a substance that has been evaluated previously is being prepared, a statement should be made to the effect that the previously published monograph has been expanded and is reproduced in its entirety below. The structure of the food additive should be normally included in a full monograph.

**2.4 Biological data** - This section contains summarized descriptions of studies that are important for assessing the safety of the food additive. Studies that provide the basis for the evaluation should be summarized in greater detail than other studies. Single paragraphs composed of one-sentence summaries may be sufficient for reporting the results of studies of limited design or minor relevance for the evaluation.

The author's conclusions should be summarized in this section. If the person who arrived at the conclusion is not identified, it is assumed that it is the author of the study. When the Temporary Adviser disagrees with the conclusions of the author, he or she should discuss the controversial issues and present his/her own conclusions as a separate paragraph. This paragraph should conclude with the Temporary Adviser's determination of the no-observed-effect level (NOEL).

If studies on more than one animal species are summarized under one heading, the studies should be grouped in such a way that studies in smaller rodent species are listed first, with larger species listed last.

When adjacent paragraphs summarize different studies under the same heading, an extra space should be left between them. However, a space should not be left between paragraphs when they both summarize the same study.

Biological data should be grouped under three headings, **Biochemical aspects**, **Toxicological studies**, and **Observations in humans**. In a full monograph, but not in a monograph addendum, if no data are available under any of these headings or under subheadings under **Toxicological studies**, except for **Special studies**, the heading should be included in the working paper with the statement "No information available."

**2.4.1 Biochemical aspects** - These studies are designed to measure the concentration/time profiles of the ingested substance and its metabolites in the various organs and tissues of the body. The Committee uses them for interpreting the toxicological studies, including elucidating the mechanism of toxicity, which facilitates the establishment of an ADI. Comparisons of biochemical data between experimental animals and humans helps determine the relevance of toxicity observed in animals, which is likely to result in a more rational assessment of risk to humans; such comparisons should be summarized at the end of this section.

The types of biochemical studies that should be summarized under each heading are given below. Human biochemical studies that fall into these categories should be included in this section. Other human studies should be included under **Observations in humans**.

#### 2.4.1.1 Absorption, distribution, and excretion

- Hydrolysis/digestion of the parent compound and its products in the mammalian gastrointestinal tract.
- The bioavailability of the unchanged compound and its hydrolysis or digestion products.
- The pattern and rate of distribution of absorbed substances within the animal.
- Mode and rate of excretion or elimination of the parent compound and its identified metabolites.

#### 2.4.1.2 Biotransformation

- Metabolism of the parent compound, if absorbed as such, and of its products if they are not normal dietary or body constituents. The toxicological importance of the identified metabolites, whether stored or excreted, should be discussed (if known). If the biochemical transformation pathway is known, a figure depicting the pathway should be included along with an identification of the species to which it applies. Such schemes are usually provided by the manufacturer and can be photocopied for inclusion in the working paper. If a metabolic scheme is not provided by the data sponsor, it should be requested.

#### 2.4.1.3 Effects on enzymes and other biochemical parameters

- The effects of absorbed substances and/or their metabolites on cellular and tissue enzyme production and morphology, chemical constitution, enzyme activity, or physicochemical state.

**2.4.2 Toxicological studies** - Summaries of toxicological studies generally comprise the bulk of a working paper. Five categories of studies on food additives should be routine; these are **acute toxicity, short-term toxicity, long-term toxicity/carcinogenicity, genotoxicity, and reproductive toxicity**. Sometimes these routine studies point toward the need for looking at particular target organs or tissues; such studies are classified as **special studies**. Appendix B provides guidance on the structure of toxicological summaries.

When the necessary information is provided, dose levels administered during the study should be given in terms of "mg of test substance/kg bw per day". If the author of the study presents administration levels in terms of "mg/animal per day", these values should be converted to mg/kg bw per day using animal weights if they are included in the report. With substances mixed in the diet, authors often express dosage levels in terms of ppm (parts per million), mg/kg feed, or percent of the substance in the diet. When feeding levels are presented in this way, they should be expressed in terms of mg/kg feed (when the lowest dose is 1000 ppm or more, it may be more appropriate to express the concentration as percent of the diet). However, when determining the NOEL, these values should be converted to equivalent mg/kg bw per day figures using appropriate conversion factors (see section 2.6). Numbers should be reduced to two significant figures, e.g. 273 ⇒ 270 or 0.273 ⇒ 0.27.

The Good Laboratory Practice (GLP) status of the study, along with the relevant authority, should be indicated. If there is no GLP certification, the Temporary Adviser should at least note whether the study was inspected by a Quality Assurance unit, as noted by the presence of a signed QA statement, and make some comment on the apparent quality of the protocol and adequacy of the methods used. In addition, whenever the author provides information on the test guideline or protocol that was followed, it should be so indicated. This information has not been provided in

working papers in the past, so the format and placement of this information has not been standardized.

Appendix B outlines the general information that should be included in summaries of toxicological studies.

**2.4.2.1 Acute toxicity** - Such studies, when properly designed and performed, identify extremely toxic compounds and provide useful information regarding target tissues and species and sex differences. When several species of both sexes are tested, information is obtained that may be useful for predicting whether toxicity is mediated through hormonal activity or whether one species should be investigated more extensively than others.

The results of acute toxicity studies that are expressed in terms of the LD<sub>50</sub> (lethal dose, median; oral, used for intramuscular intraperitoneal, or dermal administration) and/or LC<sub>50</sub> (lethal concentration, median; used for administration by inhalation) should be presented in tabular form as shown in Appendix C. When 3 or more LD<sub>50</sub> or LC<sub>50</sub> determinations by the same route in the same animal are available, the results may be expressed in a range in which the lowest to the highest values are recorded.

Other acute toxicity data important to the evaluation, such as the nature of toxicity and target tissues, may be presented in summary form below the table.

**2.4.2.2 Short-term toxicity** - Toxicological studies in which substances are administered in regularly-repeated doses over periods ranging up to one year to most small-animal species and up to two years to dogs and primates should be summarized in this section.

These studies, when properly performed, provide important information regarding the major toxic effect(s) of the test substance and its dose/response relationship. Short-term toxicity studies are often performed to ensure proper dose selection in long-term toxicity studies, and they can point toward target tissues and organs. In some cases, short-term toxicity studies can help clarify lowest-effect dose levels for effects observed in long-term toxicity studies and they can provide retrospective information that is useful for the interpretation of long-term toxicity/ carcinogenicity studies, e.g., early signs of toxicity in the kidney or liver when tumours appear in these organs after long-term administration of the test substance.

The same general format and type of information as outlined under **Long-term toxicity/ carcinogenicity** studies (section 2.4.2.3) should be included in summaries of short-term studies.

**2.4.2.3 Long-term toxicity/carcinogenicity** - Toxicological studies in which substances are administered in regularly-repeated doses over the greater part of the normal lifespan of the animal species are summarized in this section. These studies are used for detecting chronic effects that are not observed in shorter studies. Long-term studies that are designed to investigate specific effects, such as carcinogenicity, should be included in this section.

Toxicological summaries of short-term toxicity, long-term toxicity, and carcinogenicity studies should include the following information (see Appendix B):

- Species, strain, and number of each sex per dose level, including controls.
- Mode of administration.
- Duration of administration (and duration of observation period if different).
- Brief listing of the biological parameters that were studied, the techniques employed, and any other information about the design of the study considered to be relevant. When histopathological examinations were performed, the tissues that were examined should be indicated along with the identification of tissues that were of particular interest to the evaluation.

- Toxicological findings and levels at which observed. Results of relevant statistical analyses may be presented here.
- No-observed-effect levels (NOELs) for specific toxic effects.

Negative findings should be limited to general statements on survival, growth, reproduction, organ weights, tumour incidence, organ function tests, and gross and microscopic appearance of tissues.

**2.4.2.4 Genotoxicity** - Data from an appropriate battery of short-term *in vitro* and *in vivo* genotoxicity tests can be useful in elucidating the mechanism of toxicity of certain compounds. Results of these studies may also be considered when evaluating the results of rodent carcinogenicity bioassays or determining whether an *in vivo* carcinogenicity bioassay is necessary to adequately assess the carcinogenic potential of a substance. To present the data in a more understandable form and to conserve space, the results of short-term genotoxicity tests should be tabulated. Appendix D provides an example of tabular representation of such data. If it is difficult to provide details in the table on a particular study that are considered necessary, it can be described in more detail in a separate paragraph.

**2.4.2.5 Reproductive toxicity** - These studies are designed to evaluate effects on the sexuality and fertility of males and females and on developmental toxicity. **Multigeneration reproductive toxicity studies** provide general information on the effects of the test substance on gonadal function, estrous cycles, mating behavior, conception, parturition, lactation, and growth and development of the offspring until the age of weaning. **Developmental toxicity studies** are used for assessing effects on the developing organism, which may include (1) death of the developing organism, (2) a structural abnormality, (3) altered growth, and (4) a functional deficiency.

**2.4.2.6 Special studies** - Special studies are those designed to test for specific effects, such as neurotoxicity, immunotoxicity, and allergenicity. Appendix A lists, in alphabetical order, most of the special studies that will be encountered. Special studies should be listed alphabetically.

**2.4.3 Observations in humans** - Results of observations in humans are useful for assessing the relevance of the results of animal studies and for confirming ADIs. All studies dealing with humans (except for those summarized under **Biochemical aspects** or **Special studies** on effects on gastrointestinal microflora) should be included in this section, including epidemiological surveys, clinical experience, anecdotal observations, health effect studies relating to occupational exposure, reports of abuse, and volunteer studies measuring intolerance. Details such as sex, age, and general statements of physical condition should be given if important to the evaluation.

**2.5 Comments** - This section should contain short summaries of the biological findings in the studies of significance for the evaluation. The findings should be listed in the same general order as they are summarized in the main body of the working paper. All relevant NOELs should be included. When the NOEL is the same dose level (in mg/kg feed) in males and females, only one value in terms of mg/kg bw per day (the sex with the lower value) should be given. Details such as sex, age, and general statements of physical condition should be given if important to the evaluation. Issues that require resolution at the meeting should be stated either here or in a **Notes** section (see section 2.7).

This and the **Evaluation** section, which is prepared by the Member (see section 1, paragraph 2), serve as the starting point for the evaluation. These sections usually serve as the first draft for the report. Therefore it greatly increases efficiency and ensures a more orderly process if the Temporary Adviser prepares the **Comments** section in a manner consistent with the way that it will be presented in the report.

Appendix E contains an example of a report item, and should serve as a model for the preparation of the **Comments** and **Evaluation** sections.

**2.6 Evaluation** - A proposal should be provided by the Member as to whether the Committee should allocate an ADI and, if so, on what basis. *Principles for the safety assessment of food additives and contaminants in food* (WHO Environmental Health Criteria, No. 70; available at the IPCS web site ([www.who.int/pcs](http://www.who.int/pcs))) and JECFA reports on food additives should be consulted for guidance on selection of safety factors in establishing ADIs. If the Member proposes a temporary ADI, the data required to resolve the outstanding issues to permit the establishment of an ADI should be indicated. If no ADI can be established, the information that the Committee would wish to have before reviewing the compound again should be listed.

NOELs should be given in terms of mg/kg bw per day, and there are generally three ways to get there:

1. When the author of the study has presented data in these units (gavage studies) the NOEL should be reported in terms of " $\bar{x}$  mg/kg bw per day".
2. When the author has presented data in terms of parts per million (ppm), mg/kg feed, or percent in the diet and food consumption and body-weight data are available, the intake in terms of mg/kg bw per day can be calculated directly. The calculated values should ordinarily be rounded to two significant figures. The NOEL should be reported as " $\bar{x}$  ppm (or mg/kg feed or percent in the diet), equal to  $\bar{y}$  mg/kg bw per day". Only one value should be reported, which is derived from the sex that gives the lower value based on food intake and body-weight data.
3. When the author has presented data in terms of ppm, mg/kg feed, or percent in the diet and food consumption data are not available, the table in Appendix F should be used for calculating intake. The NOEL should be presented as " $\bar{x}$  ppm (or mg/kg feed or percent in the diet), equivalent to  $\bar{y}$  mg/kg bw per day".

The ADI is expressed in terms of mg/kg bw, and it should be rounded to one significant figure. The basis for the proposed ADI and safety factor should be provided.

If a temporary ADI is proposed, information required on the substance should be listed, along with a date by which time the results of the indicated studies should be submitted to WHO for evaluation.

**2.7 Notes** - This section is not included in the published monograph after the meeting. Information that the Temporary Adviser or Member would like to bring to the attention of the Committee for resolution should be included here.

If the results of a large number of studies are available, a table should be constructed that lists the NOELs and critical effects that have been identified. Such a summary table, which will not be published in the toxicological monograph, is of great value at the meeting.

## 2.8 References

**2.8.1 Citations in the text** - References in the text should be in parenthesis following the relevant summaries (e.g. Williams, 1987a; Dalidowicz & Babbitt, 1986; Dalidowicz et al., 1986; Dalidowicz, 1987).

When a report has more than two authors, the first author is followed by "et al.". It should be noted that "et al." is not underlined or italicized, "&" replaces "and", the punctuation must be correct,

and several references to the same statement (including more than one by the same author(s)) are placed in chronological order.

When more than one article by the same author(s) in any one year is cited, the year should be followed by the lower-case letters "a", "b", "c", etc.

The names of authors are not always provided. In this case, the name of the organization associated with the generation of the data, followed by the year, should be cited, for example, (IARC, 1983) or (BIBRA, 1976).

Personal communications should be cited only in the text; they should not be included in the reference list. The name of the author, the recipient, and the date should be given. If the original recipient was not the World Health Organization, the submitter of the communication to WHO should be included.

*Examples:*

(Personal communication from Prof. R. Truhaut, Director, Toxicological Research Centre, Department of Pharmaceutical and Biological Sciences, René Descartes University, Paris, France, to WHO, 1975).

(Personal communication with attachments from R. Patterson, Northwestern University, Evanston, IL, USA, to S.A. Anderson, Federation of American Societies for Experimental Biology (FASEB), Bethesda, MD, USA; submitted to WHO by FASEB).

**2.8.2 Reference list at the end of the working paper** - The layout indicated below should be used. The order in which the information is presented is particularly important.

References should be listed in alphabetical order. All authors' names and initials should be listed, the name of the first author establishing the placement in the list of references. When more than one article by the same author(s) is cited, they should be placed in chronological order and, as indicated above, the lower-case letters "a", "b", "c", etc., should be used when more than one article by the same author(s) in any one year is cited. Only initial letters are capitalized.

When the name of an author is not available, the organization associated with the generation of the data should be given first in the citation (do not use the word "anonymous").

Translated titles appear in square brackets and the original language in parentheses. Titles of articles originally in French should remain in French.

**2.8.2.1 Published studies** - References should include authors (if provided), the year of publication, the title of the article, the journal and volume number, and inclusive page numbers. Names of journals should be abbreviated according to the ISDS (International Serials Data System) List of Serial Title Word Abbreviations or otherwise given in full. The initial letter of each abbreviation is capitalized. The volume number is indicated in bold print and is followed by the issue number (if any) in parentheses. First and last page numbers must be given.

*Examples:*

Dean, I., Jackson, F. & Greenough, R.J. (1996) Chronic (1-year) oral toxicity of erythritol in dogs. *Regul. Toxicol. Pharmacol.*, **24**, S254-S260.

IARC (1983) IARC (International Agency for Research on Cancer) monographs on the evaluation of the carcinogenic risk of chemicals to humans: Miscellaneous pesticides, **30**, 329-344.

Laubstein VH & Niedegesass G (1970) [Examination of human sensitivities to nitrofurans]. *Derm Mschr*, **156**, 1-8 (in German).

**2.8.2.2 Unpublished studies** - The essential elements of unpublished studies that should be included are:

- The name of the author(s) who performed the research work, if provided.
- The year in which the experimental work was completed.
- The title of the experimental study; if the title is in a language other than English, translation of the title into English is preferred (except titles in French).
- Study number, if provided.
- An indication that the study is unpublished.
- The name of the institution at which the experimental study was performed.
- The name of the institution that submitted the report to the World Health Organization.

*Examples* - These examples provide guidance on the appropriate format to use under varying conditions, including when the names of authors are not provided, when the institution submitting the study to WHO did not perform the study, and when the institution submitting the study to WHO did perform the study.

Baker RC, Mastro CW, Kinoshita FK & Keplinger ML (1976) Acute irritation tests with a sample coded N252-C10406, Lot No. BL7668, in albino rabbits. Unpublished report No. 8530-08861 from Industrial Bio-Test Laboratories, Inc., Northbrook, IL, USA. Submitted to WHO by Uniroyal Inc., Bethany, CT, USA (validated by the Canadian Health Protection Branch).

Bailman JJ & Barber ED (1985) Evaluation of mono-t-butylhydroquinone in the CHO/HGPRT forward mutation assay. Unpublished report No. 85-0061 from Health and Environment Laboratories, Eastman Kodak Co., Rochester, NY, USA. Submitted to WHO by Eastman Kodak Co., Kingsport, TN, USA.

BIBRA (1976) A study of the haematological effects of caramel in human volunteers. Unpublished report No. 1/172/76 from the British Industrial Biological Research Association, Carshalton, Surrey, England. Submitted to WHO by International Technical Caramel Association.

Herken, H. (1961) [Pharmacological expertise on tolerance to natural and synthetic menthol.] Unpublished report from Pharmakologischen Institut der Freien Universität, Berlin. Submitted to WHO by Schering AG, Berlin, Germany (in German).

**2.8.2.3 Conference proceedings** - The following elements are necessary: Name(s) and initial(s) of author(s), the year of publication, title of paper, the word "In:" the editors of the proceedings; the full title of the conference (not abbreviated); the place and date of the conference; the place of publication; the publisher; the volume number (if any) and the page numbers.

*Example:*

Wassermann M (1984) L'étude de la toxicologie des pesticides en climat tropical In: Smith JH ed. Proceedings of the 14th international Congress of Occupational Health, Madrid, 16-21 May 1983. Amsterdam, Excerpta Medica, vol 3, 1728-1733.

#### **2.8.2.4 Books**

*Examples:*

Windholz M ed. (1983) The Merck Index: an encyclopedia of chemicals, drugs, and biologicals, 10th ed. Rahway, New Jersey, Merck and Co., Inc.

Reference to a chapter in a book should be given as follows:

Rall TW (1990) Oxytocin, prostaglandins, ergot alkaloids, and other drugs; tocolytic agents. In: Gilman AG, Rall TW, Nies AS & Taylor P eds. the pharmacological basis of therapeutics, eighth edition. New York, Pergamon Press, pp 933-953.

### 2.8.2.5 Agency reports

*Example:*

US EPA (1984) Mercury health effects update: health issue assessment. Washington, DC, US Environmental Protection Agency (EPA-60018-84-019F).

### 2.8.3 Order of entries in the list

The following rules are applied:

- a) Several papers by different authors with the same surname are listed alphabetically according to their initials.
- b) Several papers by one author are listed chronologically.
- c) Several papers by the author plus a co-author are listed alphabetically.
- d) Several papers by the author plus two or more coauthors are listed chronologically.

*Examples:*

Smith DE (1985)  
Smith JH (1983)  
Smith JH (1984)  
Smith JH & Barns MP (1986)  
Smith JH & Jones TD (1979)  
Smith JH, Jones TD, & Barnes MP (1981)  
Smith JH, Barnes MP, & Jones TD (1983)

## Appendix A

### Working paper pattern

This appendix provides the format that should be used in preparing working papers for the Joint FAO/WHO Expert Committee on Food Additives. Close adherence to this pattern will facilitate the editing of those working papers that are subsequently published as toxicological monographs.

Please adhere to the heading hierarchy shown in this appendix. The headings are indented here to clearly show the hierarchy, but they should not be indented in the text. The headings should be indented as shown in this appendix in the table of contents at the beginning of the working paper. Boldfacing should be used with all titles. The listed animal species and special studies are meant to serve as examples, not as exhaustive lists.

An extra space should be left between paragraphs when they summarize different studies; when adjacent paragraphs summarize the same study, a space should not be left between them.

#### TITLE Table of contents

1. EXPLANATION
2. BIOLOGICAL DATA
  - 2.1 Biochemical aspects
    - 2.1.1 Absorption, distribution, and excretion
    - 2.1.2 Biotransformation
    - 2.1.3 Effects on enzymes and other biochemical parameters
  - 2.2 Toxicological studies
    - 2.2.1 Acute toxicity [In tabular form; see Appendix C]
    - 2.2.2 Short-term toxicity
      - Mice* }
      - Rats* }
      - Hamsters* }
      - Rabbits* }
      - Dogs* }
      - Pigs* }
      - Monkeys* }
    - 2.2.3 Long-term toxicity/carcinogenicity
    - 2.2.4 Genotoxicity [In tabular form; see Appendix D]
    - 2.2.5 Reproductive toxicity
      - 2.2.5.1 Multigeneration reproductive toxicity
      - 2.2.5.2 Developmental toxicity
    - 2.2.6 Special studies on cardiovascular effects
    - 2.2.7 Special studies on immune responses
    - 2.2.8 Special studies on macromolecular binding
    - 2.2.9 Special studies on metabolites
    - 2.2.10 Special studies on no-hormonal effect levels
    - 2.2.11 Special studies on ocular toxicity
    - 2.2.12 Special studies on photoisomerization products
    - 2.2.13 Special studies on thyroid function
  - 2.3 Observations in humans
3. COMMENTS
4. EVALUATION
5. REFERENCES

## Appendix B

### Toxicological summaries

The summaries of experimental data form the bulk of a working paper. They are aimed at giving an overview of the essential elements of an experimental study that sometimes is several hundred pages long. Studies that are used for setting the ADI or are otherwise used for making a determination of safety should be summarized in more detail than those that are peripheral to the safety evaluation.

A good scientific summary contains the following elements:

- (a) Purpose or objective of the experiment.
- (b) Identification and purity of the substance administered.
- (c) Designation of animal species and strain employed in the study.
- (d) Number of animals in test and control groups.
- (e) Sex.
- (f) Dose levels of treatment (preferred units are mg test substance/kg of body weight per day).
- (g) Route(s) of administration.
- (h) Duration of treatment and/or the experiment, if they differ in length.
- (i) Biological parameters examined.
- (j) Effects observed (percent survival should be included in long-term toxicity/carcinogenicity studies if affected by treatment).
- (k) Author's conclusions and conclusions of the Temporary Adviser, if different from those of the author (if the conclusions are not straightforward, identify who made them).
- (l) Reference(s).

The following paragraph is an example of a typical summary of a toxicity study prepared by a Temporary Adviser in preparation for a meeting of the Committee.

#### **2.2.3      *Long-term studies of toxicity and carcinogenicity***

##### *Rats*

Groups of 45 male and 45 female inbred Wistar rats were given diets containing stevioside (purity, 85%) at 0, 0.2, 0.6, or 1.2% (equivalent to 100, 300, and 600 mg/kg bw per day) for two years. After 6, 12, and 24 months, blood was obtained from the tail vein of five male and five female rats in each dose group for haematological and clinical biochemical tests. One week later, these rats were housed in metabolism cages for urine collection and were then killed for further biochemical, pathological, and histopathological examination. All surviving animals were killed at two years. Growth, food use and consumption, general appearance, and mortality were similar in treated and control groups. The mean life span of rats given stevioside was not significantly different from that of the controls. No treatment-related changes were observed in haematological, urinary, or clinical biochemical values at any stage of the study. The incidence and severity of non-neoplastic and neoplastic changes were unrelated to the concentration of stevioside in the diet. The NOEL was 1.2%, equivalent to 600 mg/kg bw per day (Xili et al., 1992).

## Appendix C

### Tabular representation of acute toxicity data

Table 1. Acute toxicity of hydrogenated glucose syrups

Species	Sex	Route	LD <sub>50</sub> (g/kg bw)	Reference
Mouse	Male & female	Oral	16-24	Dupas, 1982a Yamasaki et al., 1973a
Mouse	Male	Intraperitoneal	11	Dupas, 1982a
Mouse	Female	Intraperitoneal	18	Dupas, 1982a
Mouse	Male & female	Intravenous	6.4-12	Dupas, 1982a Yamasaki et al., 1973b
Mouse	?	Subcutaneous	>20	Yamasaki et al., 1973b
Rat	Male & female	Oral	24-24.3	Dupas, 1982b Nishibori, 1968 Kotani & Chiba, 1968
Rat	Male & female	Intraperitoneal	13	Dupas, 1982b

## Appendix D

### Tabular representation of genotoxicity data

**Table 2. Results of assays for genotoxicity with some low-molecular-mass isoparaffins**

Test system	Test object	Chain length	Concentration	Results	Reference
In vitro					
Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C11-13	7.7-77 000 µg/ plate	Negative	Xerox Corp. (1981)
Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1983)
Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C10-13	≤10 000 µg/ plate	Negative	Phillips Petroleum Co. (1990)
Reverse mutation <sup>a</sup>	<i>E. coli</i> , WP2	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1981)
DNA damage	<i>E. coli</i> , Pol A <sup>+</sup> /A	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1981)
DNA damage	<i>E. coli</i> , Pol A <sup>+</sup> /A <sup>-</sup>	C11-13	7.7-77 000 µg/ plate	Negative	Xerox Corp. (1981)
Cell mutation <sup>a</sup>	Mouse lymphoma L5178Y cells, Tk <sup>+/-</sup> locus	C10-13	≤1000 µg/ml	Negative	Phillips Petroleum Co. (1990)
Sister chromatid exchange <sup>a</sup>	Chinese hamster ovary cells	C10-13	≤50 µg/ml	Negative	Phillips Petroleum Co. (1990)
In vivo					
Micronucleus formation <sup>b</sup>	Mouse bone marrow	C10-11	19 g/kg bw, intaperitoneally	Negative	Xerox Corp. (1983)
Dominant lethal mutation	Sprague-Dawley rat	C10-11	0, 300, 900 mg/kg feed, 6h/day, 5 d, by inhalation	Negative	Exxon Corp. (1978)

<sup>a</sup> In the presence and absence of Arochlor-induced rat liver microsomal fraction

<sup>b</sup> Killed at 48 and 72 h

## Appendix E

### Sample report item

#### ***Curdlan***

Curdlan is a linear polymer consisting of  $\beta$ -(1 $\rightarrow$ 3)-linked glucose residues. It is derived by fermentation from the bacterium *Alcaligenes faecalis* var. *myxogenes*. The Committee considered its use in food as a formulation aid, processing aid, stabilizer and thickener, or texturizer. Curdlan has not been evaluated previously by the Committee.

Information was available on the current per-capita intake of curdlan in Japan and from a scenario based on levels of use of the additive and food consumption data in the United States. This information was inadequate to assess intake completely because data on maximum use and the distribution of intake of foodstuffs that might contain the additive in various regions of the world were not provided.

In two studies, rats given  $^{14}\text{C}$ -curdlan at a dose of 20 mg/kg bw orally excreted about 80% and 40% of the administered radiolabel, respectively, as  $^{14}\text{C}$ -carbon dioxide within 24 h. In these studies, excretion in the urine represented about 3% and 1.5% of the dose and excretion in faeces about 8% and 34%, respectively. After 48 h, 100% and 80% were recovered from carbon dioxide, urine, and faeces combined. When tetracycline was given concomitantly in the drinking-water, excretion as carbon dioxide decreased by one-third, whereas excretion in faeces was increased, indicating that intestinal microflora may be responsible for the metabolism of this compound. Excretion of the radiolabel as carbon dioxide also decreased with increasing dose of curdlan, indicating more limited metabolism at higher doses. In humans, the faeces appeared to be the main pathway for excretion, except for a portion that was fully metabolized to carbon dioxide. The extent of metabolism to carbon dioxide in humans also appeared to reflect the action of intestinal bacteria: when the bacterial microflora were suppressed by pretreatment with antibiotics, very limited production of  $^{14}\text{C}$ -carbon dioxide was seen.

Curdlan given to rats at concentrations of 1, 5, or 15% in the diet had no effect on the bioavailability of calcium, magnesium, iron, zinc, copper, or manganese. The LD<sub>50</sub> value in mice and rats treated orally was > 10 000 mg/kg bw, and no abnormalities were seen at autopsy.

In short-term and long-term studies in experimental animals, the only effects of orally administered curdlan were soft stools and/or laxation, reduced body-weight gain, and increased weights of full and empty caeca due to the presence of high concentrations of 'indigestible' curdlan. In an eight-week study in mice and a four-week study in rats given curdlan at concentrations up to 300 g/kg of diet, the only effects were large faecal pellets, soft stools and/or laxation, and increased weights of full and empty caeca.

In a three-month study in rats, the lowest dose of 50 g curdlan per kg of diet was the NOEL. Growth inhibition was seen at the highest dose of 200 g/kg of diet, even though food intake was increased. Soft stools, enlarged large intestines when full, and increased weights of full and empty caeca appeared to be the major effects at 100 and 200 g/kg of diet. Dose-dependent decreases in platelet counts and protein and globulin concentrations and increased serum alkaline phosphatase activity, absolute carcass weight, and the relative weights of the adrenal and submaxillary glands were seen in males at 100 and 200 g/kg of diet. In addition, males at the highest dose had decreased serum calcium and cholesterol concentrations, and females at this dose had decreased relative pituitary weights and increased relative uterine weights. At necropsy, decreased deposition of adipose tissue was seen in the abdominal cavities of females at all doses and in males at the highest dose.

In a one-year study in dogs treated in the diet, blood-tinged, mucoid, soft stools were seen with curdlan at 150 g/kg of diet and with gelled curdlan at 400 g/kg of diet (containing curdlan at 100 g/kg, providing a final concentration of 40 g/kg of diet). Increased full and empty caecal weights were observed with curdlan at a dose of 150 g/kg of feed. The petaechial haemorrhages and mucosal ecchymosis occasionally observed in the small intestinal mucosa of dogs at all doses were considered to be unrelated to treatment.

In a lifetime study of carcinogenicity in mice, addition of gelled curdlan at 400 g/kg of diet or curdlan at dietary levels of up to 150 g/kg did not cause significant abnormalities, although decreased food consumption was seen at the highest dose of curdlan and increased food consumption with the gelled curdlan. No changes in tumour incidence were observed. In a two-year study in rats, the highest dose of curdlan (150 g/kg of diet) decreased growth and food consumption and increased the weights of full and empty caeca. The gelled curdlan had no effect.

In another two-year study, rats were exposed *in utero*. The growth of those exposed to curdlan at 150 g/kg of diet was inhibited and they showed a slight decrease in food consumption. Increased emptied caecal weights were seen in males given curdlan at 50 g/kg of diet, in females given 150 g/kg of diet, and in females given gelled curdlan at 400 g/kg of diet. Clinical chemical analyses during treatment showed increased aspartate aminotransferase and serum alkaline phosphatase activities in animals given the highest dose of curdlan or gelled curdlan. Gross and microscopic examination revealed a significantly increased incidence of benign uterine polyps in rats exposed to curdlan at 150 g/kg of diet, with incidences of 0/50 in controls, 3/50 in rats given curdlan at 10 g/kg of diet, 4/47 at 50 g/kg of diet, 7/50 at 150 g/kg of diet (significant), and 2/50 with the gelled curdlan. The authors reported that benign uterine polyps were seen infrequently in control animals; the incidences in historical controls were not available.

In a three-generation study of reproductive toxicity in rats, with two litters per generation, no effect was seen on fertility, gestation, or the viability of the pups. Parents given curdlan at 150 g/kg of diet or gelled curdlan, providing 400 g/kg of diet, showed slight growth inhibition. Food consumption was slightly decreased in parents at the highest dose of curdlan. Furthermore, female F2 parents given the high dose of curdlan or gelled curdlan in the diet had increased full and empty caecal weights. The weights of the pups in nearly all litters of dams at the high dose of curdlan were significantly decreased during lactation: in F1a and F1b litters at 14 and 21 days of age; in F2a and F3a litters at 4, 7, 10, 14, 17, and 21 days of age; in F2b litters only at day 4 of age; and in F3b litters only at day 21 of age. The NOEL for both the maternal and embryonal toxicity of curdlan was 50 g/kg of diet. Although the authors suggested that the decrease in pup weight gain during lactation at the highest dose of curdlan was due to consumption by the pups of their mothers' diet, it might also have been a treatment-related effect or a combination of consumption of the mothers' diet and an effect through the milk. In order to investigate these suggestions, a number of single-generation studies (two litters per generation) were performed in which the offspring of treated females were nursed by untreated female rats and the offspring of untreated females were nursed by treated dams. A further single-generation study was conducted with cellulose at 50 or 150 g/kg of diet. In these studies, curdlan or cellulose at 150 g/kg of diet significantly decreased pup weight gain during lactation, and transfer of pups from treated to control dams during lactation decreased this effect. When treatment of the dams with curdlan was withdrawn during lactation, the weights of the pups were comparable to those of control pups at this time.

The three-generation study of reproductive toxicity included a study of teratogenicity in the F2c litters. No embryotoxic or teratogenic effects were observed at any dose of curdlan up to 150 g/kg of feed or with gelled curdlan at 400 g/kg. In a study of teratogenicity in rabbits treated orally by gavage, no effects were seen.

Curdlan was inactive in assays for gene mutation *in vitro* in bacteria and in mouse lymphoma cells and did not induce chromosomal aberrations in hamster ovary cells. It did not induce micronucleus formation in mice *in vivo*.

No pathogenicity was observed in mice that received oral doses of live or dead cells of the curdlan-producing strain, *Alcaligenes faecalis* var. *myxogenes* NTK-u, or in mice that received intravenous, intraperitoneal, or intracerebral injection of live organisms. The strain was not cytotoxic to HeLa cells.

Curdlan was not immunotoxic in mice or rats. It did not induce skin sensitization in humans, although this study was of limited value.

In a four-week study in which six volunteers consumed up to 50 g of curdlan daily, increased flatulence was observed. One subject who consumed 50 g of curdlan per day had some diarrhoea. No evidence of toxicity was seen.

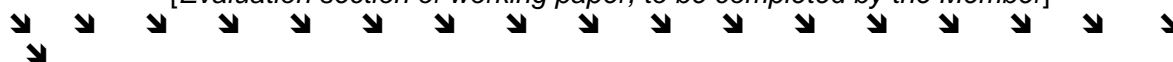


[Comments section of working paper, to be completed by the Temporary Adviser]



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[Evaluation section of working paper, to be completed by the Member]



Curdlan did not induce genotoxic, carcinogenic, or teratogenic effects or effects on reproduction. At high doses, curdlan decreased growth and/or food consumption and increased the weights of full and/or empty caeca. These effects are commonly observed after the consumption of large amounts of 'indigestible' bulking materials.

The Committee noted the significant increase in the incidence of benign uterine polyps in rats exposed to curdlan *in utero* at the high dose of 150 g/kg of diet in the long-term study. The effect appeared to be dose-related; however, uterine polyps were not observed in the long-term study in mice or in a long-term study in rats of the same strain and from the same laboratory that did not involve exposure *in utero*. These benign growths are known to occur naturally in older rats at incidences of 1–20%, depending on the study and strain. In view of the lack of genotoxicity and the structure and metabolism of curdlan, the Committee allocated a temporary ADI 'not specified' for use of curdlan as a food additive, pending the provision of information on its use and intake. Information necessary for an assessment of the intake of curdlan, including its use, the maximum and typical expected levels in the food categories in which curdlan is proposed for use, and the consumption of foodstuffs that might contain curdlan in different regions of the world, is required for evaluation in 2001.

## Appendix F

### Approximate relation of parts per million in the diet to mg/kg of body weight per day<sup>1</sup>

Animal	Weight in kilograms	Grams food consumed per day (liquids omitted)	Type of diet	1 ppm in food is equivalent to, in mg/kg bw per day <sup>2</sup>	1 mg/kg bw per day is equivalent to, in ppm of the diet <sup>2</sup>
Mouse	0.02	3		0.150	7
Chick	0.40	50	Dry laboratory chow diets	0.125	8
Rat, young	0.10	10		0.100	10
Rat, older	0.40	20		0.050	20
Guinea pig	0.75	30		0.040	25
Rabbit	2.0	60		0.030	33
Dog	10.0	250		0.025	40
Cat	2	100	Moist, semi-solid diets	0.050	20
Monkey	5	250		0.050	20
Dog	10	750		0.075	13
Man	60	1500		0.025	40
Pig or sheep	60	2400	Relatively dry grain forage mixtures	0.040	25
Cow, maintenance	500	7500		0.015	65
Cow, fattening	500	15 000		0.030	33
Horse	500	10 000		0.020	50

<sup>1</sup> Lehman, A.J. (1954). **Association of Food and Drug Officials Quarterly Bulletin** 18: 66. The values in this table are average figures, derived from numerous sources.

<sup>2</sup> 1 ppm = 1 mg/kg feed.

*Example:* What is the value in mg/kg feed and mg/kg bw per day of 0.5% substance  $x$  mixed in the diet of a rat?

**Solution**

- I. 0.5% corresponds to 5000 mg/kg feed (see below)
- II. From the table, 1 mg/kg in the feed of a rat is equivalent to 0.050 mg/kg bw per day. Consequently, 5000 mg/kg diet is equivalent to 250 mg/kg bw per day (5000 X 0.050).

