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GUIDANCE ON SUBMISSIONS FOR FOOD ADDITIVE EVALUATIONS BY THE SCIENTIFIC COMMITTEE ON FOOD

(opinion expressed on 11 July 2001)

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INTRODUCTORY REMARKS

The purpose of this document is to give guidance to petitioners and other interested parties wishing to introduce new additives into the EU market, or seeking to revise existing provisions regulating individual additives already authorised within the EU, or seeking confirmation that an already approved additive made from a new source or by a new method of production is acceptable. It gives guidance on the administrative and technical data required, on the range of toxicological tests generally required for new food additives, and on the format for formal submissions on additives (hereafter referred to as "dossiers") to the European Commission. The information submitted is needed either for the European Commission and/or for the Scientific Committee on Food.

In the European Union (EU), substances proposed as food additives may only be authorised for use if a reasonable case of technological need can be demonstrated, if they present no hazard to the health of consumers at the level of use proposed, and provided they do not mislead the consumer.[1] The European Commission is required to consult the Scientific Committee on Food (SCF) prior to submission of any proposals for legislation on food additives where these may have an effect on health. Although evaluation of the safety of proposed and permitted food additives is carried out by the SCF, the Committee generally plays no role in assessing aspects such as technological need, benefit to the consumer, or whether the use of an additive would mislead consumers. The mandate of the SCF is to advise the Commission on "scientific and technical questions concerning consumer health and food safety associated with the consumption of food products and in particular questions relating to toxicology and hygiene in the entire food production chain, nutrition, and applications of agrifood technologies, as well as those relating to materials coming into contact with foodstuffs, such as packaging".[2]

Petitioners wishing to make a food additive submission to the European Commission are advised to consult both this main Guidance Document and the accompanying Annex.

Petitioners should note that competent authorities in Member States may request copies of any dossier submitted to the Commission and should ensure that this can be supplied without delay upon request.

Member States should also be mindful of the guidance in this document when making a submission to the EU on any additive admitted provisionally for marketing and use within national territories under Directive 89/107/EEC (Article 5). Member States should ensure they submit a full dossier together with a written evaluation carried out in their country on the safety in use of the additive.

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1 Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption. Official Journal of the European Communities L 40, 11.02.1989 p. 27 – 33. (Article 1.2 includes definition of a food additive)

2 Commission Decision 97/579/EC of 23 July 1997 setting up Scientific Committees in the field of consumer health and food safety. Official Journal of the European Communities L 237, 28.08.1997, p. 18.

PROCESS OF EVALUATION BY THE SCF OF A SUBMISSION

ON A FOOD ADDITIVE

A description of the process of evaluation by the SCF of a submitted dossier is shown in Appendix 1.

DOSSIERS

INTRODUCTION

The preferred format for dossiers is set out below.

Dossiers are archived by the Commission and may be made available to outside parties. Petitioners should therefore indicate if any parts of the dossier are confidential business information, bearing in mind that, in the interest of transparency, confidentiality should be restricted to those aspects for which it is strictly necessary. Petitioners are requested to retain copies of their petition.

SUMMARY DOCUMENT

The summary should follow the same order as described for the main dossier and normally not exceed 15 pages.

The nature of the proposed additive, its source, method of manufacture, proposed use, etc should be outlined and the main findings in any toxicological tests should be summarised. Petitioners are invited to present their own conclusions as to the likely safety-in-use of the substance, drawing attention to any unusual features in the data presented.

PART I ADMINISTRATIVE DATA

1. Name of the petitioner (company, organisation, etc), address and other means of communication, e.g. telephone, telefax, e-mail.

2. Name of the manufacturer(s) of the substance (if different from above), address and other means of communication, e.g. telephone, telefax , e-mail.

3. Name of the person responsible for the dossier, and means of communication, e.g. telephone, telefax, e-mail.

4. Date of submission of the dossier.

5. Table of contents of the dossier.

PART II TECHNICAL DATA

1. Identity of substance

1.1. Chemically defined single substance (e.g. sorbic acid, sodium ascorbate, propyl gallate, glycerol, etc)

– Chemical name according to IUPAC nomenclature rules

- CAS number (if this has been attributed)
- Synonyms, trade names, abbreviations
- Molecular and structural formulae
- Molecular weight
- Spectroscopic data which allow the identification of the substance
- Purity in percentage; method of determination; data printout (chromatograms, spectra, etc)
- Impurities: nature, percentage and methods of determination
- Description of physical state
- Solubility
- Other data that the petitioner considers may be useful to identify the substance

1.2. Chemically defined simple mixtures (e.g. sorbitol syrup, lecithin, etc)

- Chemical name if any
- CAS number (if this has been attributed)
- Synonyms, trade names, abbreviations
- Constituents of the mixture and proportion of each component of the mixture
- Molecular and structural formulae of the components of the mixture
- Molecular weight of the components of the mixture
- Spectroscopic data which allow the identification of the mixture, e.g. IR, UV, NMR, MS, etc
- Purity in percentage; method of determination; data printout (chromatograms, spectra, etc)
- Impurities: nature, percentage and methods of determination
- Description of physical state
- Solubility
- Other data that the petitioner considers may be useful to identify the substance

1.3. Complex mixtures (e.g. waxes, mineral hydrocarbons, anthocyanins, caramel, xanthophylls, etc)

- Source materials
- Chemical name if any
- CAS number (if this has been attributed)
- Synonyms, trade names, abbreviations
- Chemical description and composition (if measurable)
- Description of physical state
- Degree of purity; principle of the method used for determining overall purity
- Impurities: nature, percentage and methods of determination
- Solubility
- Other data that the petitioner considers may be useful to identify the substance (e.g. spectra, chromatograms, etc)

1.4. (Bio)polymers (e.g. agar, alginate and xanthan gums, pectins, starches, modified starches, celluloses, polyvinylpyrrolidone, etc)

- Chemical name if any
- CAS number (if this has been attributed)
- Synonyms, trade names, abbreviations

- Chemical description
- Chemical formula and ranges of prevailing molecular weight
- Degree of substitution, percentages of substituted groups (where appropriate)
- Description of physical state
- Solubility
- Description of identification tests
- Assay: percentage and mode of calculation
- Degree of purity (if applicable)
- Impurities: nature, percentage and methods of determination
- Other data that the petitioner considers may be useful to identify the substance

2. Microbiological characteristics

2.1 Where microbes, living or dead, are present in the final product, information on their possible pathogenicity or toxicogenicity and their effect in the gut should be described.

3. Proposed chemical and microbiological specifications

3.1 The proposed specifications should be submitted in a format modelled on recent EU or other internationally accepted specifications. Where the proposed specifications differ from any already existing JECFA or other internationally recognised specification, these specifications should be set out alongside the proposed new specification, and any differences pointed out.

4. Manufacturing process

The following information should be included:

4.1 Method of manufacture (e.g. the source, the process by which the raw materials are converted to the finished product), production controls and quality assurance.

4.2 For chemically synthesised substances, factors such as reaction sequence, side reactions, purification and preparation of the product to be commercialised which may assist in determining likely impurities and their influence on the toxicological evaluation.

4.3 For substances extracted from natural sources, information on extraction procedure(s).

5. Methods of analysis in food

Information should be provided on:

5.1 Analytical methods for the determination of the substance and its degradation products (where relevant) in the foodstuff of which the

substance is to form part. Methods should be given in full except where the analytical methods used are well established and may be given by reference only.

6. Reaction and fate in food

Information should be provided on:

6.1 The stability and any degradation products or reaction products appearing as a result of processing, storage and preparation of foods containing the substance.

6.2 Any possible effect on nutrients.

7. Case of need and proposed uses

Information should be provided on:

7.1 Technological need; intended use; benefit to the consumer.

7.2 The quantity to be added to specific foods (intended use levels or maximum use level) and the residues in food.

7.3 Investigations on the efficacy of the substance for the intended effect at the level proposed.

See also Appendix II to this document.[3]

8. Exposure

8.1 Information should be provided on known or anticipated human exposure to the proposed additive from food, including amount (e.g. maximum and average intake or exposure), frequency and other factors influencing exposure. Information should also be given on any other sources of human exposure to the same substance (e.g. from drinking water, consumer products, etc.)

8.2 The above exposure calculations should be explained, including any assumptions made. Where possible, information should be provided on consumption of the foods in which the additive is used or intended to be used, including variations affecting particular sections of the population (e.g. by age, sex, disease, etc).

[3] Annex II of Council Directive 89/107/EEC, Official Journal L 40 , 11/02/1989 p. 27 - 33

9. Additives produced by microbiological processes

9.1 In the special case of additives produced by microbiological processes, the following additional information should be provided:

9.1.1 History of the microorganism(s) used. If it has a history of safe use in food, then 9.1.3 and 9.1.4 are not required.

9.1.2 Information from previous human exposure to the product or its source.

9.1.3 Pathogenicity of the organism(s).

9.1.4 Toxicology of the organism(s).

9.1.5 Specification of the production process (see 4).

9.1.6 Specification of the end product (see 3).

9.2 In cases where the microorganism(s) and/or their metabolites remain in the final product, information should also be provided on:

9.2.1 Safety of the microorganism(s) and/or their metabolites in the final product (see 2.1).

9.2.2 Any special procedures for preparation and cooking to ensure safety.

9.3 In the case of additives produced by microbiological processes using genetically modified microorganisms (GMMs), information should also be provided on:

9.3.1 Source of the transgenic DNA and its effect on the properties of the host organism(s).

9.3.2 Genetic stability of the GMMs.

9.3.3 Probability of transfer of inserted genetic material to human gut flora and its likely consequences.

9.3.5 Ability of the GMMs to survive in and colonise the human gut.

10. Additives produced from genetically modified organisms

10.1 In the case of additives produced from genetically modified organisms, information should also be provided on the genetically modified organism(s) in accordance with the guidance given by the Scientific

Committee on Food.[4] (For gentically modified microorganisms, see 9 above.)

11. Information on existing authorisations and evaluations

11.1 Information on any existing national authorisations and evaluations and/or evaluations by other bodies should be provided.

[4] SCF (1997). Recommendations concerning the scientific aspects of information necessary to support applications for placing on the market of novel foods and novel food ingredients. Part I. Opinion expressed on 7 June 1996. Scientific Committee for Food (Thirty-ninth Series). Part II. Opinion expressed on 13 December 1996. Scientific Committee for Food (Fortieth Series). Part III. Opinion expressed on 13 December 1996. Scientific Committee for Food (Fortieth Series). Commission of the European Communities, Directorate-General Industry, Luxembourg. Note that new guidance on novel foods and novel food ingredients is being prepared by EC Scientific Committees during 2001 and petitioners should ensure they consult the latest guidance.

PART III TOXICOLOGICAL DATA

1. General framework for the toxicological evaluation of food additives

If the technological need and value to consumers of a proposed food additive have been established, it is necessary to evaluate the implications for the health of the consumer due to the presence of that additive in food. The SCF first issued Guidelines for the Safety Assessment of Food Additives in 1980.[5] Many of the principles articulated in that document are still applicable today. However, since that time, a number of other guidance documents have been published on the principles for assessment of food additive safety, including by the Joint FAO/WHO Expert Committee on Food Additives (JECFA),[6] and there is now considerable international consensus on these general principles. It is therefore not the intention to reiterate these here, but to outline a framework of core tests required by the SCF for safety evaluation of a food additive. Accordingly, the guidance given here and in the accompanying Annex replaces that given by the SCF in 1980 on the toxicological tests generally required for additives.

The aim of toxicological testing is to determine whether the substance, when used in the manner and in the quantities proposed, would pose any appreciable risk to the health of consumers. Such testing should provide not only information relevant to the average consumer, but also relevant to those population groups whose pattern of food consumption, physiological or health status may make them vulnerable, e.g. young age, pregnancy, diabetes, etc. No fixed programme of testing is laid down, but a general framework covering core tests and other tests is given, which should enable petitioners to determine what information is required to establish the safety-in-use of the food additive. The studies required will depend on the chemical nature of the additive, its (proposed) uses and levels of use in food, whether it is a new additive or a re-examination of an existing additive. In addition to laboratory tests, it may be possible to use human data derived from medical use, occupational epidemiology, or specific studies on volunteers (e.g. on absorption and metabolism) or on critically exposed groups. However, it is recognised that for new food additives, a safety evaluation generally relies on experimental data largely derived from investigations in laboratory animals. If the biological action of a substance has been ascertained qualitatively and quantitatively in a range of tests on laboratory animals, the likely effects on man can then be estimated by careful extrapolation.

In general, this guidance is intended to apply to the evaluation of a proposed new food additive, or to the re-evaluation of an already approved food additive, directly incorporated into food and fulfilling a defined technical purpose. It does not apply to flavouring substances, substances migrating into food from food packaging materials, or novel foods, on which the SCF has issued separate guidance, nor does it apply to other food contaminants, either naturally occurring or man-made. It is however recognised that

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5 Scientific Committee for Food (1980). Guidelines for the Safety Assessment of Food Additives. Reports of the Scientific Committee for Food (Tenth series). Commission of the European Communities, Luxembourg EUR 6892.

6 IPCS/JECFA (1987) Environmental Health Criteria 70: Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization, Geneva.

many of the tests used for the safety evaluation of food additives are also useful for the toxicological evaluation of other types of food chemicals.

2. Study protocols

Studies on toxicity, kinetics and metabolism of food additives should be conducted using internationally agreed protocols. Test methods described by OECD[7] or in European Commission Directives [8,9] are recommended. It is advisable to ensure the most up-to-date edition of any test guideline is followed. Use of any methods differing from internationally agreed protocols should be justified. Protocols for special studies differing from standard tests should be developed on a case-by-case basis.

To ensure mutual recognition by Member States of the data submitted, studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directive 87/18/EEC[10] and accompanied by a statement of GLP compliance. The scope of GLP requirements was extended to include food additives in 1988 under Council Directive 88/320/EEC[11]. Adequate explanation should be provided for divergence from these principles. Studies conducted prior to the introduction of GLP for food additives may be considered, particularly if they relate to older substances or additives already permitted for use in the EU.

Petitioners are reminded that Council Directive 86/609/EEC[12], on the protection of animals used for experimental and other scientific purposes, requires that care is taken to avoid unnecessary use of animals. Studies carried out should be those necessary to demonstrate the safety of an additive and planned in accordance with the principles of reduction, refinement and replacement. However, at this point in time, assuming adequate

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7 OECD (2000). OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, Eleventh Addendum and subsequent revisions.

8 Commission Directive 87/432/EEC of 3 August 1987, adapting to technical progress for the eight time, Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities No L 239,21.8.1987, p1.

9 Commission Directive 92/69/EEC of 31 July 1992, adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities No L 383, 29.12.1992, pA/1.

10 Council Directive 87/18/EEC of 18 December 1986 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. Official Journal of the European Communities No L 15, 17.1.1987, p29.

11 Council Directive 88/320/EEC of 9 June 1988 on the inspection and verification of Good Laboratory Practice (GLP). Official Journal of the European Communities No L 145, 11.6.1988, p 35.

12 Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities No l 358, 18.12.1986, p 1.

human data are not available, *in vivo* studies using experimental animals from species relevant to humans are still needed in order to assess possible risks to humans from the ingestion of food additives. There are some exceptions to this (e.g. assessment of genotoxic potential by *in vitro* studies) and alternative validated methods for other endpoints in toxicity, involving fewer or no animals, are being developed. Studies submitted using alternative methods would be considered on a case-by-case basis.

3. Toxicological section of the dossier

Any proposed food additive should normally undergo a comprehensive examination for potential toxicological effects before its safety-in-use can be accepted. Petitioners should therefore submit the range of studies recommended below as a core set and should consider whether any other types of study might also be appropriate.

There may be circumstances, depending on the substance and its uses, under which it is considered that a full core set is not necessary (e.g. substances which are normal constituents of the diet or of the body, or may be metabolised to such; toxicological data on close homologues or other structure activity considerations; intake considerations). Or, as specified tests are completed, it may be possible that a decision on safety can be taken in the light of the results obtained without further tests. Or it may be decided that tests other than the usual tests are more appropriate.

The reasons for carrying out any unusual studies should be stated, as should the reasons for not submitting a study of a type that might be expected. All the important results obtained, favourable or unfavourable, should be presented and discussed and the original study reports submitted in order to allow independent, critical appraisal. Petitioners should also submit any other existing, relevant data on the substance, including copies of published papers.

3.1. Core studies

The core studies normally required for evaluation of the safety of a food additive are set out below. The detailed considerations underlying these core toxicological requirements have been elaborated in the Annex to these guidelines.

a) Metabolism/Toxicokinetics

Information on metabolism and toxicokinetics should normally be provided on a new additive. The design of metabolism and toxicokinetic studies should be flexibly adapted to the particular substance being tested. Not all aspects may need to be investigated in every case. In principle, whole animal studies using single and repeat dosing are needed. *In vitro* studies can also contribute useful information.

b) Subchronic toxicity

Any new additive should normally be tested in subchronic toxicity studies, preferably in which the additive is given via the diet, in two laboratory species, usually a rodent and a non-rodent, for a period of at least 90 days. Preceding feeding studies conducted for 14 or 28 days can provide an indication of target organs and help in selection of appropriate doses for 90-day studies, but studies of shorter duration than 90-days are generally not sufficient, by themselves, for evaluation of potential subchronic toxicity.

c) Genotoxicity

Any new additive should normally be tested for genotoxicity in order to assess its mutagenic and carcinogenic potential. In general a battery of three genotoxicity tests is required, comprising:

i. A test for induction of gene mutations in bacteria.

ii. A test for induction of gene mutations in mammalian cells *in vitro* (preferably the mouse lymphoma *tk* assay).

iii. A test for induction of chromosomal aberrations in mammalian cells *in vitro*.

Positive results in any of the above in vitro tests will normally require further assessment of genotoxicity in vivo.

d) Chronic toxicity and carcinogenicity

Any new additive should normally be tested for chronic toxicity and carcinogenicity in studies, preferably in which the additive is given via the diet, in two laboratory species, usually rat and mouse, covering the majority of the lifespan of the animals, generally 24 months in the rat and 18 or 24 months in the mouse. Combined chronic toxicity/carcinogenicity studies are acceptable.

e) Reproduction and developmental toxicity

A new additive should normally be tested in reproduction and developmental toxicity studies. A multigeneration reproduction study, including assessment of endpoints relevant to endocrine disrupter potential, should be conducted in one laboratory species, usually rat, and comprise at least two generations and one litter per generation. Administration of the test substance should normally be in the diet.

Developmental toxicity studies should be conducted in two laboratory species, usually a rodent and a non-rodent, such as rat or mouse and rabbit. Administration of the test substance should normally be via the diet or by oral gavage and cover not only the period of embryogenesis but continue to the end of gestation in order to ensure detection of, for example, endocrine disrupter potential.

In order to ensure that a new additive does not affect postnatal development and function, including neurological function and behaviour, physical, functional and

behavioural development of animals exposed from at least the beginning of embryogenesis through to weaning should be studied. This can be done as a separate study or as part of a multigeneration and/or developmental toxicity study.

3.2 Other studies

In addition to the core studies, other studies may also be helpful or necessary for certain substances, depending on aspects such as chemical structure or class, uses, and known or predicted toxicological properties. Decisions on whether other studies are needed should be taken on a case-by-case basis. Examples of other areas of investigation which might be appropriate include, but are not limited to: immunotoxicity, allergenicity, intolerance reactions, neurotoxicity, human volunteer studies, predictive mechanistic studies and special studies to explore in more depth toxicological effects observed in core studies.

Further discussion on the relevance, scope and use of other studies, including those mentioned above, is provided in the Annex to these guidelines.

There are also other toxicity studies that are not required for evaluation of the safety of food additives, but which may have been conducted for other purposes, such as worker safety (e.g. acute toxicity, irritation and sensitisation studies). If such studies are available, they should be submitted as they may provide useful background information.

4. Data reporting

The data reported for standard toxicological tests should, as far as possible, follow the recommendations for data reporting given in the relevant OECD guidelines[13]. Petitioners are reminded that for each study performed it should be stated whether the test material conforms to the proposed or existing specification. If it does not conform then the specifications of the test material should be given and it should be indicated whether this is representative of the substance intended for the market.

5. Review of results and conclusions

For each study, the significant findings should be highlighted, together with the no-adverse-effect level, if one has been determined, and any other relevant information. Where effects are seen only at high doses/concentrations, the relationship between the exposures giving rise to effects and likely human exposure from use of the substance as an additive should be discussed.

This section should also seek to interpret the data and draw conclusions. The reasons for disregarding any findings should be carefully explained. Where necessary, the conclusions should include an interpretation of the significance of the findings in terms of possible mechanisms of any effects observed, a discussion of whether these are relevant to humans and, if so, the possible significance of the extrapolation of such

[13] Ninth Addendum to the OECD Guidelines for the Testing of Chemicals. OECD, Paris, February 1998.

findings to humans. References to effects (or lack of effects) from known human exposure should be given; evidence from recorded experience for occupational or therapeutic exposure, for example, may be informative. The overall evaluation of potential human risk should be made in the context of known or likely human exposure, including that from other sources.

PART IV REFERENCES AND REPORTS

1. List of references

References should be quoted as follows:-

i. Published data

Journals: Author(s) (full list including all names and initials), title of article, journal, volume number, page numbers, date.

Books: Author(s), title of chapter/book, editor(s) (if relevant), publisher, location, date, page numbers (if relevant).

ii. Unpublished data

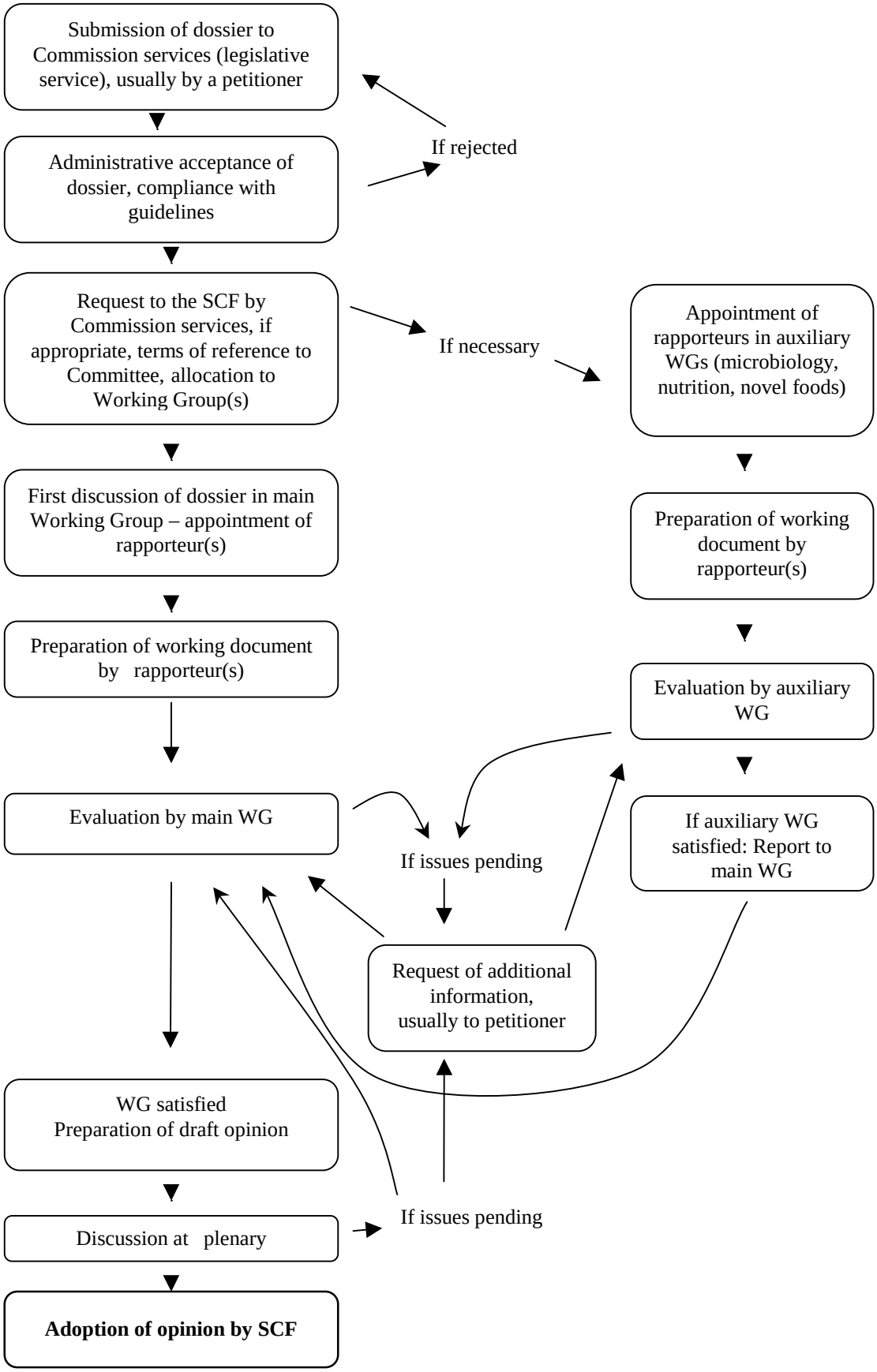
Name of petitioner, title of report, report reference, name of investigator(s) (if any), name of laboratory, address of laboratory, date.

2. Appended papers and study reports

Copies of key papers from the references cited which might be needed for an independent safety evaluation should be submitted with the dossier.

Copies of all unpublished study reports should be submitted in full. Summaries of unpublished studies are not acceptable.

APPENDIX I: FLOWCHART OF THE PROCESS OF EVALUATION BY THE SCF OF A DOSSIER ON A FOOD ADDITIVE



APPENDIX II: GENERAL CRITERIA FOR THE USE OF FOOD ADDITIVES

Note: The information below is quoted *verbatim* from Annex II of Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption. Official Journal of the European Communities, L 40, 11.2.89, p.27.

1. Food additives can be approved only provided that

   – there can be demonstrated a reasonable technological need and the purpose cannot be achieved by other means which are economically and technologically practicable,

   – they present no hazard to health of the consumer at the level of use proposed, so far as can be judged on the scientific evidence available,

   – they do not mislead the consumer.

2. The use of food additives may be considered only where there is evidence that the proposed use of the additive would have demonstrable advantages of benefit to the consumer, in other words it is necessary to establish the case for what is commonly referred to as 'need'. The use of food additives should serve one or more of the purposes set out from points (a) to (d) and only where these purposes cannot be achieved by other means which are economically and technologically practicable and do not present a hazard to the health of the consumer:

   a) to preserve the nutritional quality of the food; an intentional reduction in the nutritional quality of a food would be justified only where the food does not constitute a significant item in a normal diet or where the additive is necessary for the production of foods for groups of consumers having special dietary needs;

   b) to provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs;

   c) to enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that this does not so change the nature, substance or quality of the food as to deceive the consumer;

   d) to provide aids in manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of the use of faulty raw materials of undesirable (including unhygienic) practices or techniques, during the course of any of these activities.

3. To assess the possible harmful effects of a food additive or derivatives thereof, it must be subjected to appropriate toxicological testing and evaluation. The evaluation should also take into account, for example, any cumulative, synergistic or potentiating

effect of its use and the phenomenon of human intolerance to substances foreign to the body.

4. All food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information.

5. Food additives must at all times comply with the approved criteria of purity.

6. Approval for food additives must:

    a) specify the foodstuffs to which these additives may be added and the conditions under which they may be added;

    b) be limited to the lowest level of use necessary to achieve the desired effect;

    c) take into account any acceptable daily intake, or equivalent assessment, established for the food additive and the probable daily intake of it from all sources. Where the food additive is to be used in foods eaten by special groups of consumers, account should be taken of the possible daily intake of the food additive by consumers in those groups.

ANNEX

CONTENTS

I. INTRODUCTION

This Annex to the main document "Guidance on Submissions for Food Additive Evaluations by the Scientific Committee on Food" discusses the detailed considerations underlying the core toxicological studies required by the Scientific Committee on Food (SCF) for food additive submissions to the European Commission. Core studies are those normally required for evaluation of the safety of a food additive or a substance proposed as a food additive and they are set out in the main guidelines entitled "Guidance on Submissions for Food Additive Evaluations by the Scientific Committee on Food". In addition to the core studies, this Annex also discusses the relevance, scope and use of other studies which may be necessary or helpful to establish safety for certain substances.

Petitioners wishing to make a food additive submission to the European Commission are advised to consult both the main document "Guidance on Submissions for Food Additive Evaluations by the Scientific Committee on Food" and this accompanying Annex to the main document.

II. CORE STUDIES

a) Metabolism and toxicokinetics

Metabolism and toxicokinetic studies provide data on absorption, distribution, metabolism and elimination (ADME) of the test substance. ADME data describe important characteristics of a substance that should be taken into consideration when assessing the safety of a food additive. They are useful for (a) deciding on the extent of toxicological testing (e.g. reduced testing may be possible for substances not absorbed or metabolised to normal dietary or body constituents), (b) selecting appropriate test species, dose levels and duration of toxicity studies, (c) interpretation and evaluation of the other toxicity data, and (d) understanding the mechanism of toxicity.

The design of metabolism and toxicokinetic studies must be flexibly adapted to the particular substance being tested. Not all aspects may need to be investigated in every case. In principle, whole animal studies using single and repeat dosing are needed. These will enable determination of gastrointestinal absorption and overall elimination rates, any changes in the kinetic behaviour of the substance with repeated administration, and, if comparative studies are available, evaluation of species differences. They may also provide information on other aspects relevant to toxicity, such as placental transfer, induction or inhibition of metabolising enzyme systems and reversible or irreversible binding to tissue sites and carrier proteins. It should be noted that *in vitro* studies, employing enzymes, subcellular organelles, cell cultures and perfused organs, can also contribute useful information in the investigation of metabolic pathways, mechanisms of toxicity, effects on enzymes and other specific aspects.

For substances that are complex mixtures, conventional metabolism and toxicokinetic studies may not be feasible. Other ways of studying bioavailability and, if appropriate, tissue distribution or accumulation should be sought.

Toxicokinetic information in humans is also of considerable value for a number of reasons, including confirmation of the validity of the animal models used. Any available information on the substance itself or on chemically related substances should be

submitted. Petitioners are also encouraged to develop such information on a proposed additive where possible (see III.e. Human volunteer studies).

b) Subchronic toxicity

The major objective of subchronic toxicity studies is to determine the toxicological profile of the test substance following repeated administration to experimental animals over a prolonged period of time. Such studies provide information on the target organs, on the nature and severity of any effects, and on the dose-response relationships. They should allow determination of the dose at which adverse effects found at higher dose levels are no longer observed, i.e. the no-observed-adverse-effect level. They are invaluable for estimating the appropriate dose levels for chronic toxicity studies and they can provide indications for the need for additional studies on particular effects, such as neurotoxic or immunological effects.

Any new additive should normally be tested in subchronic oral toxicity studies in two laboratory species, usually rodent and non-rodent, for a period of at least 90 days. Preceding feeding studies conducted for 14 or 28 days can provide an indication of target organs and help predict appropriate doses for 90-day studies, but, in general, studies of shorter duration are not sufficient, by themselves, for evaluation of potential subchronic toxicity. If possible, animal species should be used in which the metabolic pathways and kinetic parameters of the same or analogous substances are similar to those in man (see (a) above).

According to the relevant OECD Guidelines (1, 2), the studies should include measurements of food consumption and body weight, haematological examinations, clinical biochemical determinations in plasma or serum and, in non-rodents, urinalysis. In rodents, urinalysis is only optional, but should be performed when there is reason to expect any effects on urine parameters. In addition to a detailed gross necropsy and the determination of organ weights, a full histopathology is necessary.

c) Genotoxicity

Food additives should be evaluated for genotoxicity in order to assess their mutagenic and carcinogenic potential. The objectives of genotoxicity testing include both the detection of germ cell mutagens, because of their possible involvement in the aetiology of human heritable genetic defects, and the detection of somatic cell mutagens, because of their involvement in neoplastic transformation and other diseases. Nowadays, it is acknowledged that each of the three levels of mutation, namely gene, chromosome, and genomic mutations (numerical chromosome changes leading e.g. to aneuploidy) may play a role in inherited disorders and cancer. For cancer in particular, extensive experimental data on human tumours demonstrate the activation of oncogenes through point mutation or translocation, and/or the inactivation of tumour suppressor genes by loss of heterozygosity or other mutational events (3).

The basic battery of tests

At present, no single validated test method can provide information on all the above-mentioned genetic end-points and so it is necessary to test each chemical in several assays to get full information on its genotoxic potential. In the case of chemicals for which wide human exposure is expected (e.g. food additives, pesticides,

pharmaceuticals), several testing strategies have been proposed over the years which comprise three to four tests at the gene and at the chromosome level, sometimes also including an *in vivo* assay (4-7).

In general, the SCF requires a battery of three *in vitro* genotoxicity tests for food additives, though positive results require confirmation in subsequent *in vivo* studies. The required studies are:

1. A test for induction of gene mutations in bacteria.

2. A test for induction of gene mutations in mammalian cells *in vitro* (preferably the mouse lymphoma tk assay).

3. A test for induction of chromosomal aberrations in mammalian cells *in vitro*.

For test methods, it is recommended that OECD protocols be used. The OECD has recently updated six relevant guidelines and introduced a new one. The revised guidelines provide guidance for the conduct of tests *in vitro* for the detection of gene mutations in bacteria (8) and in mammalian cells (9), for the detection of chromosomal aberrations in mammalian cells (10), and for the conduct of *in vivo* assays for chromosomal aberrations (11) and micronuclei (12) in rodent bone marrow, and chromosomal aberrations in spermatogonia (13). The new guideline concerns the *in vivo/in vitro* assay for the detection of UDS in rat liver (14). The same updated test procedures are proposed for adoption as official test methods by the European Union.

As far as tests for gene mutation in mammalian cells are concerned, the forward mutation assay at tk locus in mouse lymphoma L5178Y cells is the preferred test system. Compared to gene mutation systems at hemizygous loci (e.g. the HPRT system), the mouse lymphoma assay allows the detection of a wider range of genetic events, including those leading to loss of heterozygosity. Thus this system may show an intrinsically greater sensitivity in screening for potential genotoxins.

While the above battery of tests is considered as the core set for food additives, there may be circumstances under which it may be justified to deviate from the core set. In such cases the scientific justification for deviation from the core set should be provided. A reduced battery or even no testing could, for example, be acceptable in cases where intrinsic physico-chemical properties are indicative of lack of activity.

In other cases, supplementary modified *in vitro* or *in vivo* assays may be required, especially when metabolic or toxicokinetic data indicate that *in vitro* tests are of limited significance (e.g. because of the existence of metabolic pathways involving extra-hepatic metabolism or cytochrome isoenzymes poorly represented in liver S9).

Aneuploidy

Consideration should be also given to the possible disturbance of chromosome segregation leading to aneuploidy. The updated OECD protocol for metaphase analysis requires the scoring and recording of polyploidy, which may give some clues on this end-point. Further relevant information on the aneugenic potential of a substance can be given by the bone marrow micronucleus test, possibly coupled with methods enabling the detection of centromeres or kinetochores in micronuclei, which allow the detection of chromosome loss due to various mechanisms. For this reason, it is suggested that the

micronucleus test should be the method of choice in the assessment of cytogenetic effects *in vivo*.

In vivo testing

*In vivo g*enotoxicity assays play a key role in the follow-up of positive results *in vitro*. In any case, positive results *in vitro* should trigger an investigation of the potential expression of genotoxicity in somatic cells *in vivo* and, in the event of positive results in somatic cells, in germ cells also. However *in vivo* studies at the germ cell level should be considered on a case-by-case basis. In general, for *in vitro* genotoxins, a negative outcome in *in vivo* bone marrow cytogenetics and/or liver UDS assays indicates that the potential of the chemical to mutate germ cells is low or absent. On the other hand, local genotoxic effects (e.g. in the upper gastrointestinal tract) cannot be ruled out solely on the basis of inactivity in bone marrow or liver, especially for directly acting, electrophilic molecules. In these situations, expert judgement should be made on a case-by-case basis, considering the available information on chemical reactivity, as well as absorption, excretion and metabolism. Tests to investigate the possible formation of DNA adducts may, for example, be appropriate.

Future developments

The Committee is aware of current developments that may alter the approach to genotoxicity testing in the future. For example, a protocol for an *in vitro* micronucleus test is currently being evaluated for inclusion in OECD guidelines for genetic toxicology testing. This test procedure might be considered for inclusion in test batteries in the near future, as an alternative to the *in vitro* chromosomal aberration assay. This provides an efficient tool for the detection of spindle disturbance which may lead to chromosome loss, in addition to clastogenicity. For the time being, however, cytogenetic damage should be evaluated according to the standard *in vitro* method for metaphase analysis.

Recently, the International Conference on Harmonisation (ICH) for human pharmaceuticals has proposed the use of the mouse lymphoma assay as a surrogate for both gene and chromosomal mutation tests (6). However the use of this test for chromosomal aberration seems premature in view of the relatively limited supporting database (15, 16), and the unclear weight of protocol variations (e.g. microwell versus soft agar protocols, short versus long treatments) on the performance of the assay (17). Results from ongoing collaborative studies on L5178Y cells will offer the opportunity for reconsideration of this test in the future.

Other test procedures, such as the *comet* assay, or tissue specific mutations in transgenic animals may provide additional useful information on the genotoxic and carcinogenic potential of a food additive. However, some of these methods are still under validation and, for the time being, they should be applied with caution in hazard identification.

It is clear that, while very informative methods can be employed at present for the detection of genotoxic potential, the field is continuing to evolve. The need for periodic revision of these guidelines, in the light of state-of-the-science and technical progress, is envisaged.

d) Chronic toxicity and carcinogenicity

Chronic toxicity

The major objective of a chronic toxicity study on a food additive is to provide information on gross and histopathological changes other than neoplasia in organs and tissues, and changes in blood, urine and serum chemistry following long term exposure, via an appropriate, oral route. Such studies may reveal new effects not evident in subchronic studies, or they may confirm effects observed in subchronic studies at the same or perhaps lower doses. Chronic toxicity studies are often pivotal in defining critical no-observed-adverse effect levels for setting Acceptable Daily Intakes for additives.

Chronic toxicity may be studied by itself, using the relevant OECD protocol (18). Alternatively, the use of a combined protocol to study chronic toxicity and carcinogenicity in the same experiment will often be appropriate in the testing of food additives (19). The most commonly used species for chronic studies are rodents. In a few circumstances, it may be appropriate to conduct long term toxicity studies in other species, such as the dog or non-human primate, if the nature of the toxicity observed, the toxicokinetics or the procedures required necessitate the use of such species.

Microscopic examination should cover all organs and tissues in the body. It is however acceptable to examine control and top dose animals only for microscopic changes, provided no significant pathological changes are observed in the top dose group. Tissues from lower dose groups should always be retained in case further examination is required. It is desirable to include clinical chemistry, urinalysis and haematological measurements on subgroups of animals at intervals of 3,6 and 12 months of age and at termination of the study, though interpretation of the more variable results obtained at the end of the animals' lifespan can be difficult. If such sampling is not done on satellite groups of animals killed at interim intervals, care needs to be taken to ensure that the general health of animals so sampled is not compromised if they are to continue to the end of the study.

Carcinogenicity

Proposed food additives should normally be evaluated for carcinogenicity in laboratory animals. The objective of a carcinogenicity study on a food additive is to observe test animals for the development of neoplastic lesions following exposure for the majority of their life span, by an appropriate oral route of administration.

The animal models used for carcinogenicity testing should be biologically appropriate for the assessment of possible human risk. However, the selection of test species is usually limited, by practical considerations, to laboratory rats and mice. The OECD protocol for carcinogenicity studies (20) has not undergone any significant changes during recent years. Since chronic toxicity as well as carcinogenicity information is normally required for food additives, a combined protocol for studying chronic toxicity and carcinogenicity in the same experiment is recommended in order to maximise the information provided from the animals used (19). *In utero* exposure is not required unless specific considerations suggest otherwise. In selecting the highest dose for testing, particular attention should be paid to the recommendations of the OECD (19, 20) that it should be sufficiently high to elicit minimal toxicity without substantially altering the normal life span due to effects other than tumours. This Maximum Tolerated Dose (MTD) may, for example, be a dose that causes a reduction in body weight gain no greater than 10%.

Doses higher than the MTD are not generally appropriate for extrapolation to humans with respect to carcinogenicity (21). For food additives, which are relatively non-toxic chemicals, it may prove impossible to identify such a dose level in a meaningful way. In the case of food additives given via the diet, the highest dose should normally not exceed 5 % of the diet, in order to avoid nutritional imbalances. This upper dose is acceptable even if no toxicity is produced.

In the event of a carcinogenic response being demonstrated, additional mechanistic information together with good data on toxicokinetics are usually essential for risk assessment, both with respect to extrapolation to humans and possible determination of a threshold for non-genotoxic carcinogens. These considerations emphasise the need to carefully examine interim results from a long-term study as soon as they become available, in case the study protocol needs modifying or extra studies need to be planned, in order to provide mechanistic or toxicokinetic information.

Future developments in carcinogenicity

There is an ongoing discussion in the scientific community about whether the use of both rodent species is necessary (22) and thus whether a long-term study in the rat supplemented by a short- or medium-term *in vivo* rodent test, such as a model of initiation-promotion, or a carcinogenesis model using neonatal or transgenic mice, may be an acceptable alternative to life-span bioassays in a two rodent species (23); or whether the male rat and female mouse could be used as an alternative to a standard two species, two sex bioassay (24); or whether a life span study in a single rodent species in combination with short-term genotoxicity tests and mechanistic information may be sufficient (22). Reduced bioassay testing may well be sufficient for the detection of genotoxic carcinogens as these are more often found to give rise to tumours in more than one species, in more than one sex and at more than one site, compared with non-genotoxic carcinogens (25, 26). However, to optimise the likelihood of detecting non-genotoxic carcinogens, testing in more than one species may be more appropriate. Until there is an international consensus on newer approaches for carcinogenicity risk assessment, both sexes of rats and mice should normally be used for the testing of food additives, unless specific considerations suggest otherwise.

e) Reproduction and developmental toxicity

Reproduction

The major objective of a reproduction study is to provide information about effects on male and female libido, potency and fertility, on the female's ability to carry pregnancy to term, on maternal lactation and care of the young, on the prenatal and postnatal survival, growth and development of the offspring, on the reproductive capacity of the offspring and to identify histologically any major target organs for toxicity (including reproductive organs) in the parents and offspring. Information about the functional and behavioural development of the offspring is also now considered relevant and these aspects are discussed further below.

Since potential human exposure to most food additives spans a lifetime, a reproduction study on an additive should normally be a multigeneration study conforming to a modern protocol, which includes assessment of endpoints relevant to endocrine disrupter potential. The study should be conducted in one laboratory species, usually the rat, and include at least two generations and one litter per generation. In certain circumstances

(e.g. poor reproduction in controls or possible treatment-related effects that are unclear), a second litter may be required. Such designs also permit the detection of any potential for effects to increase in successive generations. The test substance should normally be administered in the diet with continuous exposure of parental and offspring generations.

More focused investigation of male reproductive capacity is not routinely required but may occasionally be needed, depending on the structure of the chemical and the outcome of multigeneration and other studies.

Prenatal developmental toxicity

The major objective of a prenatal developmental toxicity study (formerly called a teratogenicity study) is to identify any potential to cause lethal, teratogenic or other toxic effects on the embryo and fetus, by examination for embryonic and fetal resorptions or deaths, fetal weight, sex ratio, and external, visceral and skeletal morphology. An additive should normally be tested in two laboratory species, usually a rodent and a non-rodent, such as rat or mouse and rabbit. Other species may be acceptable or needed in certain circumstances. Administration of the test substance should cover not only the period of embryogenesis but continue up to sacrifice of the dams, which are killed for examination of the fetuses one or two days before the end of pregnancy. This extension of the dosing period, beyond just embryogenesis as was previously required, is to ensure the detection of, for example, certain endocrine disrupter properties. The test substance may be administered in the diet or by gavage. A developmental toxicity study carried out as a satellite phase within a multigeneration study may be acceptable for the rodent study.

Postnatal developmental toxicity

Exposure to chemicals prenatally via the mother and postnatally via maternal milk may also impair postnatal development and function, including neurological function and behaviour. Investigation of these aspects can be carried out as part of the multigeneration study (e.g. by selecting one or more males and females per litter for testing), and/or as an additional satellite phase in the developmental toxicity study, or as a completely separate study. Adverse effects of this nature may occur in animals which otherwise appear overtly morphologically normal. Physical, functional and behavioural development in animals exposed from at least the beginning of embryogenesis through to weaning should be studied. Although there has been considerable experience of this type of testing with pharmaceuticals, no single prescriptive approach is advocated. An appropriate strategy would include a broad range of observations designed to assess development, function and behaviour during both the early postnatal phase and adulthood. Relevant observations may, for example, include pup body weight, preweaning physical and functional developmental landmarks including reflex development, the onset of sexual maturity as measured by vaginal opening in females and cleavage of the balanopreputial gland in males, sensory and locomotor function, and some indication of cognitive ability (learning and memory) (see also section III.d. Neurotoxicity).

Additives for infant formula: a special case

Standard toxicity testing protocols do not adequately model artificial feeding in the neonatal phase. Neonates also take some time in achieving adequate detoxification mechanisms. For these reasons special strategies have been developed, which are set out in the SCF opinions on the applicability of the ADI Acceptable Daily Intake for food

additives to infants (expressed on 17/9/1998) and on additives in nutrient preparations for use in infant formulae, follow-on formulae and weaning foods (expressed on 7/6/1996).

In the case of an additive which is proposed for use in infant formula and whose structure is such that an evaluation cannot be made from existing data, a special strategy may be required. The dosing regime for the test animal should adequately model the human exposure situation with respect to dose, exposure to the parent compound, and species differences in the timing of neurological development and other developmental parameters. This may not necessarily be achieved if exposure is only indirect via dosing of the mother and passage of the test substance and/or its metabolites into maternal milk. It may therefore be necessary to conduct a study in which the test substance is administered orally, directly to the offspring, from birth through to weaning. Postnatal survival, growth, development, function and behaviour of the offspring should be examined. In some circumstances, studies on other species (e.g. primates or pigs) may need to be considered for adequate modelling of the human situation.

III. OTHER STUDIES

Other studies may be relevant and useful for establishing the safety of an additive and several such types of study and general approaches are discussed below. This should not however be taken as a complete list of all the types of study that may be undertaken.

In considering the first 3 sections covering immunotoxicity, allergy and food intolerance, it is useful to have in mind the following definitions:

> **Immunotoxicity** describes the undesired effects of chemicals on the immune system that may undermine the effectiveness of its responses against foreign substances and pathogens.
>
> **Allergy** is an immunopathological response to exposure mainly to a protein or a protein hapten conjugate.
>
> **Food intolerance** is an adverse reaction to food that can present with the same symptoms as food allergy, but which is not, or not directly mediated through the immune system and may involve several mechanisms.

a) Immunotoxicity

Introduction

The major objective of immunotoxicology studies is the detection and evaluation of undesired effects of chemicals on the immune system and its functions that may undermine the effectiveness of immune responses against foreign substances and pathogens. Substances with immunotoxic properties will be potentially immunotoxic to everyone in the general population. Toxic responses may occur when the immune system acts as a passive target of chemical insults, leading to altered immune function. Chemically induced toxicity, in which the immune system is the target, can result in an increased incidence of infectious disease and certain tumour diseases. It can also result in exacerbations of allergic and autoimmune disease in genetically predisposed individuals (27).

The body is protected from invading pathogens by non-specific resistance mechanisms, such as lysozyme, interferon and complement systems, phagocytosis of particles by leukocytes and macrophages. In addition, protection is mediated by specific immune responses. The immune response is a complex of processes that result from the interaction of cells of the immune system with antigen, forming a defence against infectious agents, foreign tissue grafts and certain neoplastic cells. A fundamental characteristic of immunity is the establishment of memory: the first contact with the antigen (e.g. an infectious organism) imprints specific information. Upon a second contact with the same antigen a so-called secondary immune response will occur that is usually faster and may be more pronounced than the primary response. The specific and non-specific defence systems act in concert. There are two arms within the specific immune system that operate more or less independently of each other, although many interrelations exist. The first is cell-mediated immunity, which comprises those reactions which operate by specifically sensitised T-lymphocytes and are transferable by these cells. Different types of those reactions are the classical cell-mediated defence immunity (against fungi, viruses, bacteria), delayed-type of hypersensitivity, rejection of tumours

and foreign tissues such as transplants. The other arm is constituted by humoral immunity, which operates by antibody-producing plasma cells (B-lymphocytes). This type of immunity is transferable by serum and includes antibody-mediated protective immunity, ~~and~~ immediate hypersensitivity (anaphylaxis), cytotoxic and complex mediated type~~)~~ reactions (27). Evaluation of the integrity of these various aspects of the normal immune response forms the basis of an assessment of the potential immunotoxicity of chemicals.

Testing for immunotoxicity

There is a wide array of methods to assess immune function available for most animal species. However, the usefulness of several of these tests for the detection and evaluation of direct immunotoxic effects of chemicals in food is limited or their validity is not yet established. A tiered approach is preferred in order to make suitable selection from the numerous assays possible.

For the safety evaluation of food additives, among other tests, a repeat-dose 90-day toxicity study in rats is normally required. In such studies, assessments which probe the immune system are already included. Studies in accordance with OECD guideline 408 (28) include assessments of circulating white blood cell differentiation and weights and histopathology of lymphoid organs and tissues, such as thymus, spleen, mesenteric lymph nodes, popliteal lymph nodes and Peyer's patches. Bone marrow cellularity is also investigated. This type of assessment is often referred to as Tier I immunotoxicity testing. If Tier I screening reveals immunotoxic effects not judged to be secondary to other toxic or nutritional effects of the chemical and at a dose level relevant in relation to other toxic effects, second-tier studies are indicated. Tier II testing comprises a variety of *in vivo*, and *ex vivo in vitro* assays, to assess cell-mediated and antibody-mediated immunity (humoral), macrophage function, natural killer cell activity, and host resistance in experimental infection models. Host resistance models assess several aspects of the functioning of the immune system. Apart from functional endpoints, infection models also may include evaluation of histological lesions. Such immune function assays may be especially useful for establishing no-observed adverse effect levels and for risk assessment.

Since the current core studies may not identify all potential immunotoxic compounds, further amendments to OECD guidelines on repeated-dose toxicity tests are currently being discussed such as the addition of some simple immune function tests. One such test is the assay of specific antibody titers after sensitisation to sheep erythrocytes. Antibody titers result from degradation of erythrocytes, presentation of erythrocyte antigens to T-cells, and T-cell dependent maturation of B-cells and antibody production by plasma cells. In other words, all major components of the immune system are involved in the eventual antibody titer, making this assay an adequate tool to probe for potential disturbance of this system.

b) Allergenicity

Allergy is an immunopathological response to exposure mainly to a protein or a protein hapten conjugate. Only certain (atopic) individuals with a genetic predisposition will respond in this way. The majority of adverse reactions to food additives are non-immunologically based (see III.c on Food intolerance, below). However, it is possible that allergic reactions to food additives could occur, particularly those that contain

proteins or peptides. At present there are no validated animal models to establish the potential of food components (including additives) to cause allergic reactions in susceptible individuals following oral exposure. Animal models which may eventually prove useful are being studied but general testing for such effects is not currently feasible. Detection of such effects is only possible at present through post-marketing surveillance.

Methods to investigate dermal (contact) or inhalatory routes of sensitisation, performed primarily for occupational risk assessment are available but their relevance, if any, to oral allergenicity is unclear. Information on workers who develop allergic responses to food additives during synthesis or manufacturing could be a useful alert. Some assessment of allergenic potential may also be possible from information on chemical structures and their relation to known allergens, together with information on their resistance to proteolysis.

c) Food intolerance

Food intolerance is an adverse reaction to food that can present with the same symptoms as food allergy, but which is not, or not directly mediated through the immune system and may involve several mechanisms These include enzyme deficiency , intrinsic pharmacological activity of the food component or release of endogenous mediators following direct interaction with basophilic and mast cells (pseudoallergy). It may be expressed, for example, as asthma, eczema, urticaria or gastrointestinal reactions, depending on the substance and the individual. Some of the mechanisms are well understood. However, because of the diversity of reactions, the importance of individual susceptibility (including genetic susceptibility, reactivity of the nervous system, etc), there are no simple animal models to screen for intolerance. Methods of investigation in individuals thought to be susceptible have been discussed in an earlier SCF report (29).

d) Neurotoxicity

Adult neurotoxicity

If a core study, such as the 90-day oral toxicity study, or other information, such as screening results, structure-activity relationships or physicochemical properties, indicates neurotoxic potential in a test substance, this will require further investigation using a tiered approach. The test strategy should not be rigid, but should be determined on a case-by-case basis. However, some general principles exist. The study initially indicating neurotoxic potential is considered a Tier 1 test. The following strategy is recommended for further neurotoxicity testing.

Tier 2 tests, such as the OECD Guideline "Neurotoxicity Study in Rodents" (30), are broad, exploratory tests but designed specifically to evaluate the nervous system; the aim is to support or eliminate the suspicion of neurotoxicity, but not to identify the exact nature of the disturbance. Information from previous studies is used to improve the design with respect to dose selection. Tier 2 studies are normally carried out at lower doses than those used in Tier 1 to avoid confounding of results by general toxicity.

Tier 3 comprises specialised studies on substances that are judged as definite neurotoxicants or substances for which strong evidence for neurotoxicity exists. The aim is to elucidate mechanisms in order to extrapolate from animals to humans and to further characterise and complete the risk assessment.

Tier 2 and Tier 3 studies focus on motor activity and function, sensory perception and cognitive function in laboratory animals. Affective and personality effects are signs in humans that cannot readily be observed in animals with present methods. Assessment of behavioural effects should be carried out and then considered alongside any appropriate neuropathological, neurophysiological and neurochemical evaluations, since structural or chemical changes in the nervous system cannot always be interpreted without a behavioural correlation. Validated methods exist to assess the main categories of behaviour. Valuable guidance on testing strategy and selection of test methods can be found in OECD Draft: "Neurotoxicity Guidance Document, 2000"(31).

Developmental neurotoxicity

The potential effect of chemicals on the development of the brain during embryonic organogenesis and subsequently during infancy may also need to be considered. Triggers for such studies might include the observation of abnormalities in brain morphology, function or behaviour in core developmental toxicity studies, the observation of neurotoxic effects in adults, or knowledge from structure-activity considerations that the substance is a potential neurotoxicant. An OECD Guideline for developmental neurotoxicity is in preparation (32).

e) Human volunteer studies

Introduction

Much useful information could be gained from human studies conducted before or after the marketing of a food additive. Similarly, experience gained from the investigation of the safety of human therapeutic agents may be applicable in some circumstances to human studies with food additives.

Indications for human volunteer studies

Human studies should not be used to establish general safety of a food additive. Studies of food additives in humans should only be proposed if there are adequate data from animal and other related studies to demonstrate the likely safety in humans at the proposed level of exposure. Any proposed studies should have clear scientific objectives and adequate protocols, include provisions for review in the event of occurrence of unexpected results, and comply with the relevant ethical and legal standards. These include approval by an appropriately constituted review or ethical body, adherence to the principles of informed consent by volunteers, and the maintenance of records that are open to inspection.

Types of human volunteer studies

Human volunteer studies are generally of two types, absorption, metabolism and elimination studies and tolerance studies. Other special studies e.g. on allergy, behaviour or cognitive function may sometimes be appropriate. Human volunteer studies may also be indicated when knowledge is required about special subgroups of the general population who may be genetically predisposed to low tolerance or particularly exposed to certain additives.

Studies of the absorption metabolism and elimination of additives in humans would greatly enhance the predictive value of the traditional chemical, biochemical and

toxicological investigations in laboratory animals used to demonstrate safety. Comparison of the results of such human studies with those obtained in laboratory animals enables validation of the database acquired in animal experiments and the detection of any significant differences between animals and humans, which can be of importance for the interpretation of unusual or adverse findings. Gastrointestinal absorption may be followed by determination of blood levels at intervals after administration, giving some indication of bioavailability. Information on kinetics and metabolism following absorption can be obtained from blood and urine measurements.

Human studies are particularly appropriate for investigating tolerance of a substance or a food. They may be appropriate, for example, for investigating symptoms which cannot be studied in animals (e.g. headaches, gastrointestinal discomfort). They may include physical examination, blood chemistry, haematology, urine analysis and organ function tests. At the same time monitoring for any adverse reactions, and recording their nature, frequency, intensity and dose relationship should be carried out.

A number of publications contain useful information on the conduct of clinical studies (33-39).

f) *In vitro* studies other than genotoxicity studies

Introduction

In recent years there has been considerable development of new toxicological methods not based on the use of animals, which have become known as "alternative methods". In the field of food additives a number of alternative testing strategies are available, but none has yet been validated in terms of reliability and relevance (40). However, progress towards the goal of suitable alternative tests continues, with validation procedures supervised by the European Centre for the Validation of Alternative Methods (ECVAM, Joint Research Centre, Ispra, Italy), which is officially charged by the European Commission with this task (41, 42).

In vitro systems are currently used for two main purposes: as a method of screening for certain biological and toxicological effects, and for mechanistic studies. These differ with respect to the approach adopted. The screening approach can give indications about the biological effects of substances and, as consequence, on their hazard at the cellular level. The other approach is to use cellular and subcellular systems to conduct predictive mechanistic studies at an early stage in the development of a safety testing strategy for an additive. This enables subsequent toxicity studies to be more focused on particular endpoints or aspects of function that are predicted as likely to be affected. More commonly, mechanistic studies are conducted at a later stage in testing, to investigate mechanisms of effects demonstrated in animal studies.

Several attempts have been made, at national and international levels, to validate *in vitro* methodologies as alternatives to *in vivo* studies. So far, with the exception of areas like genetic toxicology, phototoxicity and corrosivity, for which validated *in vitro* methods are now internationally accepted for regulatory purposes, assays on other useful toxicological endpoints have yet to be further developed and/or validated.

Screening for biological and toxicological effects

Both biological and non-biological systems can be used and, although representing very simplified models, they show much potential and useful information can be derived from

them on the possible hazards of chemicals. Among the first, cell cultures have been widely used for toxicological examination of chemicals and, more recently, for natural toxins (either bacterial or derived from plants) or for compounds of natural origin (43). It is also possible to rank or classify new substances on the basis of their cytotoxicity with respect to a reference list, thus contributing, at least in a preliminary phase, to the evaluation of the risk that they may represent for human health (44).

For acute and subacute assays it is not always necessary to test substances on primary cells derived from target organs. A good strategy is that of using cell lines, not necessarily differentiated, of tumoral origin. These models are generally well-characterised and easy to handle, thus assuring good reproducibility of the results. Different cell lines can be used in a battery which can also identify the most sensitive lines. Ideally, cells of human origin should be utilised in order to obtain information on the species of interest (45). Different endpoints inherent to basal cell functions can be investigated, such as cell viability (necrosis and apoptosis), cell proliferation, membrane damage, morphology and metabolic alterations. Most of these methods can be miniaturised and automated, thus allowing the processing of many compounds and concentrations at the same time.

Mechanistic studies

For mechanistic studies, the focus has been on biotransformation studies, which can be successfully performed, mainly on isolated hepatocytes, but also on hepatoma cell lines and on genetically engineered cells (46-49). Cells from other organs and tissues also display different levels of metabolic capability *in vitro* (50), which may be useful for toxicological investigation of particular target organs. *In vitro* models also offer the possibility of pharmacodynamic studies to evaluate the effects of substances and/or their relevant biotransformed products at cellular and subcellular level, including information on the precise site(s) of action and details of mechanism.

Specific investigations on alterations in sophisticated cellular functions, such as apoptosis and the regulation of gene expression, can be performed on primary cultures or freshly isolated cells from target organs (51, 52) or on established cell lines which are able to express in culture at least some of the specialised properties they have in the organism (53). Among them the most widely used are neuronal cells, kidney cells and keratinocytes. Information about effects on cellular and subcellular structures, can be integrated, for example, with studies on regulation of gene expression and/or metabolism to help identify the endogenous structures/molecules which are likely to be targets for interactions. The identification of such targets should, in turn, aid in prediction of the potential impact of the additive on main metabolic pathways, including transcription, protein synthesis and protein sorting. This can provide key information for understanding the possible adverse effects of additives and their relative compatibility with pharmacological or other treatments or other chemical exposures.

Modelling the gastrointestinal tract

In the case of food additives, modelling of the gastrointestinal tract may also be helpful. Many models of the gastrointestinal tract are available, but among the most relevant are enterocytes, either as primary cultures or established cell lines. With these cells it is possible to study effects on very specialised functions and gain an in depth understanding of the mechanism of action or absorption of chemicals. When the cells are able to

differentiate in culture, it is also possible to study effects on various stages of differentiation and on the activities which are expressed in late culture. In particular, some cells, mainly established cell lines derived from intestine, are able, when cultivated on permeable filters, to polarise and to form a typical epithelial barrier very similar to that present in the organ *in vivo*. In these experimental conditions, once the tight junctions between the cells are fully developed, it is also possible to specifically study the effect of substances on the apical (luminal) and on the basolateral (serosal) side of the cells. A number of cell lines have been successfully used, including Caco-2 cells (54-56), HT-29 cells (57, 58) and T 84 cells (59).

g) Special studies

Under certain circumstances, it may be necessary to perform special studies to explore in more depth some particular toxic effect seen in the initial studies, to give some insight into mechanisms of toxicity, to explore the possibility of particular effects signalled by structure-activity considerations, or to establish whether an effect is species-specific. Such studies may be carried out on the initiative of the petitioner and submitted as part of the initial dossier, or they may be required by the Committee following its initial evaluation of the dossier. In cases where special studies are appropriate or required, it may be advisable to consult the Committee for views on aspects which should be taken into consideration in design of the studies.

h) Acute toxicity

Acute toxicity studies are not required for the safety assessment of food additives. However, if such studies have been conducted for other purposes then they should be submitted. These and other range finding studies may provide information on target organs for toxicity. If acute studies are conducted, petitioners are encouraged to use methods that require fewer animals or do not use death as an endpoint, such as OECD Guidelines 420 or 423 (60, 61).

i) Skin and eye irritation and skin sensitisation

These tests are not relevant and not required for the safety evaluation of food additives.

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