WHO FOOD ADDITIVES SERIES: 22

# Toxicological evaluation of certain food additives

Prepared by
THE 31st MEETING OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

IPCS · International Programme on Chemical Safety

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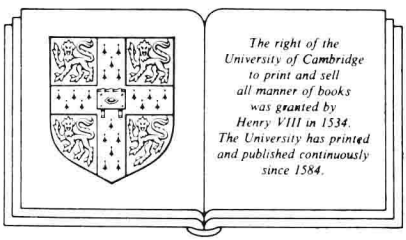
**The 31st Meeting of the Joint FAO/WHO Expert Committee on Food Additives**

*Geneva 16-25 February 1987*

Published on behalf of

The World Health Organization




**CAMBRIDGE UNIVERSITY PRESS**

Cambridge

New York New Rochelle

Melbourne Sydney

Published by the Press Syndicate of the University of Cambridge
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
32 East 57th Street, New York, NY 10022, USA
10 Stamford Road, Oakleigh, Melbourne 3166, Australia



First published 1988

Printed in Great Britain at the University Press, Cambridge

*Library of Congress cataloging in publication data available.*

*British Library cataloguing in publication data*
Joint FAO/WHO Expert Committee on Food Additives,
*Meeting (31st : 1987 : Geneva).*
Toxicological evaluation of certain food additives and contaminants.
1. Food additives — Toxicology 2. Food contamination
3. Food poisoning
I. Title II. World Health Organization

ISBN 0 521 36928 2

The preparation of this document was supported by the International Programme on Chemical Safety (IPCS), Geneve, Switzerland.

## PREFACE

The monographs contained in this volume were prepared by the thirty-first Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Geneva, Switzerland, 16-25 February 1987. These monographs summarize the safety data on selected food additives by the Committee. Generally, the compounds on which monographs were prepared are those on which substantial safety data exist. The data reviewed in these monographs form the basis for acceptable daily intakes (ADIs) established by the Committee.

The thirty-first report of JECFA has been published by the World Health Organization in the WHO Technical Report Series (No. 759). The participants in the meeting are listed in Annex 3 of the present publication and a summary of the conclusions of the Committee is included as Annex 4.

Specifications established by the thirty-first meeting of JECFA have been issued separately by FAO under the title *Specifications for the identity and purity of certain food additives*, FAO Food and Nutrition Paper, No. 38. These toxicology monographs should be read in conjunction with the specifications and the report.

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives are listed in Annex 1.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States, and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, regarding the safety of food additives and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The toxicological monographs contained in this volume are based upon working papers that were prepared by temporary advisers in advance of the thirty-first JECFA meeting. A special acknowledgement is given to those who prepared these working papers: **Dr C.L.Galli**, Professor of Experimental Toxicology, University of Milan, Milan, Italy; **Dr S.I.Shibko**, Associate Director for Regulatory Evaluation, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA; and **Dr Ronald Walker**, Professor of Biochemistry, University of Surrey, Guildford, Surrey, England.

Many proprietary unpublished reports are referenced. These were voluntarily submitted to the Committee by various producers of the food additives under review and in many cases these reports represent the only safety data available on these substances. The temporary advisers based the working papers they developed on all the data that were submitted, and all these studies were available to the Committee when it made its evaluations.

From 1972 to 1975 the toxicology monographs prepared by Joint FAO/WHO Expert Committees on Food Additives were published by WHO in the WHO Food Additives Series; after 1975 this series became available only in the form of unpublished WHO documents provided on request by the Organization. Beginning with the 1985 monographs, they are published by Cambridge University Press, which should ensure that these monographs are more widely known and available.

The preparation and editing of the monographs included in this volume have been made possible through the technical and financial contributions of the Participating Institutions of the International Programme on Chemical Safety (IPCS), which support

the activities of JECFA. IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, which is the executing agency. One of the main objectives of IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in the IPCS concerning the legal status of any country, territory, city, or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by those organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological data on the compounds reported in this document should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

## CONTENTS

# ENZYME PREPARATIONS

## ENZYME PREPARATIONS

Problems in evaluating the safety of enzymes in food processing were discussed at the fifteenth, eighteenth and twenty-ninth meetings of the Expert Committee, when principles relating to their evaluation were elaborated (Annex 1, references 26, 35, and 70). At its present meeting, the Committee reaffirmed those principles, which have been consolidated in Annex III of "Principles for the Safety Assessment of Food Additives and Contaminants in Food" (Annex 1, reference 76).

For the purpose of toxicological evaluation, the enzyme preparations under present consideration were grouped into the following classes:

- Class III - Enzymes derived from Aspergillus oryzae;
- Class IV - Enzymes derived from Aspergillus niger; and
- Class V - Enzymes derived from Trichoderma reesei, Trichoderma harzianum, Penicillium funiculosum, Aspergillus alliaceus.

The guidelines established by JECFA for these classes of enzymes provide a basis for the toxicological studies required for their evaluation.

At the twenty-ninth meeting the Committee concluded that, when enzyme preparations from either class IV or class V are added directly to food but not subsequently removed, an acceptable daily intake should be established to ensure that levels of the enzyme preparations in food are safe. In order to evaluate the information

received on the estimate of the amount of enzyme preparations used in the toxicological studies and levels of consumption resulting from their use in food, the Committee adopted the concept of enzyme total organic solids (TOS), which is defined as follows: % TOS = 100-(A+W+D), where A = % ash, W = % water, and D = % diluent and carrier (Ad hoc Enzyme Technical Committee, 1981; Pariza & Foster, 1983). This concept overcomes the problem that enzyme preparations of different activities and forms were used in the toxicological studies. It also takes into account that most of the organic solids in this fraction are not the enzyme per se.

In establishing acceptable daily intakes for the enzymes in classes IV and V, the Committee noted that the animal feeding studies were primarily of short-term duration. It, therefore, concluded that it would be appropriate to use a safety factor greater that the usual 100.

## REFERENCES

Ad hoc Enzyme Technical Committee (1981). The 1978 enzyme survey, summarized data, National Academy of Sciences/National Research Council/Food and Nutrition Board, Committee on GRAS List Survey, Phase III, National Academy Press, Washington, D.C.

Pariza, M.W. & Foster, E.M. (1983). Determining the Safety of Enzymes used in Food Processing, J. Food Protection, 46: 453-468.

## ENZYMES DERIVED FROM ASPERGILLUS ORYZAE

### EXPLANATION

Enzymes from this source were considered at the fifteenth meeting of the Committee (Annex 1, reference 26), at which time a decision on the ADI was postponed because of concern that one of the known metabolites of A. oryzae is β-nitropropionic acid, which was suspected of carcinogenic potential. Later, at the eighteenth meeting of the Committee, a lipase derived from this organism was considered (Annex 1, reference 35). It was determined at that time that there was no information to substantiate the concern for the potential carcinogenicity of β-nitropropionic acid, and that analyses of foods have shown that the metabolite is present in very few foods and then only in minute amounts. The present Committee was also informed that A. oryzae varieties are used in certain parts of the world in the preparation of foods.

### α-AMYLASE (E.C. 3.2.1)

#### BIOLOGICAL DATA

**Biochemical aspects**

No information available.

## Toxicological studies

### Acute toxicity

| Animal | Route | $LD_{50}$ | Reference |
|---|---|---|---|
| Mouse (Novo Strain) | Oral | > 20 g/kg b.w. | Novo, 1971a |

### Short-term studies

Rats

Three groups, each containing 5 male and 5 female SPF Wistar rats, were maintained for 3 weeks on diets containing 0, 0,5, or 5% of the enzyme preparation. Only minor differences were observed among the groups in body-weight change and food intake. At termination of the study, haematologic measurements, organ-weights analyses, and gross post mortem examinations showed no compound-related effects (Novo, 1971b).

In another study, two groups, each containing 10 male and 10 female ARS Sprague-Dawley rats, were fed diets containing 5 or 10% of the test enzyme (equivalent to 3.5 or 7 g enzyme/kg/b.w./day) for 90-94 days. A control group of 20 male and 20 female rats was maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar among animals in the test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 in all groups. At the end of the study, haematologic parameters, organ-weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al., 1972a).

A similar study was performed with carbohydrases from A. oryzae (α-amylase and amyloglucosidase), prepared under different culture conditions. No compound-related effects were reported (Gavin et al., 1972b).

### Long-term studies

No information available.

### Observations in man

No information available.

## COMMENTS

Short-term studies on α-amylase from A. oryzae did not reveal any adverse effects. Based upon its lack of toxicity and the fact that A. oryzae varieties are used in the preparation of foods, this enzyme was considered to be acceptable for use in food.

## EVALUATION

### Level causing no toxicological effects

Rat: 10% in the diet, equivalent to 7 g/kg b.w./day.

### Estimate of acceptable daily intake

Acceptable for use in food when used according to good manufacturing procedures.

## REFERENCES

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Bowers, S., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972a). Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972b). Carbohydrase from A. oryzae. Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

Novo (1971a). Acute toxicity of fungamyl to mice. Unpublished report from Novo Industri A/S, Bagsvaerd, Denmark. Submitted to WHO by Novo Industri A/S, Bagsvaerd, Denmark.

Novo (1971b). Three week oral toxicity study of fungamyl in rats. Unpublished report BSi/BS from Novo Industri A/S, Bagsvaerd, Denmark. Submitted to WHO by Novo Industri A/S, Bagsvaerd, Denmark.

## PROTEASES (E.C. 3.4.21.14; 3.4.23.6)

### BIOLOGICAL DATA

#### Biochemical aspects

No information available.

#### Toxicological studies

**Acute toxicity**

No information available.

**Short-term study**

Rats

Two groups of 10 male and 10 female ARS Sprague-Dawley rats were fed diets containing 5 or 10% of the test enzyme preparation (equivalent to 3.5 or 7 g enzyme preparation/kg b.w./day) for 90 to 94 days. A control group of 20 male and 20 female rats were maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar in animals in the test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 in all groups. At the end of the study serum clinical chemistry parameters, organ weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al, 1972).

**Long-term studies**

No information available.

#### Observations in man

No information available.

### COMMENTS

A short-term study in rats on a protease preparation from A. oryzae did not reveal any adverse effects. Based on its lack of toxicity and the fact that A. oryzae varieties are used in the preparation of foods, this enzyme was considered to be acceptable for use in food.

## EVALUATION

### Level causing no toxicological effect

Rat: 10% in the diet, equivalent to 7 g/kg b.w./day.

### Estimate of acceptable daily intake

Acceptable for use in food when used according to good manufacturing procedures.

## REFERENCE

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Bowers, S., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972). Protease from Aspergillus oryzae. Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

## ENZYMES DERIVED FROM ASPERGILLUS NIGER

### EXPLANATION

A. niger is a contaminant of food and was not considered in the same light as those organisms regarded as normal constituents of food. It is necessary to show that the strains used in enzyme preparations do not produce mycotoxins.

Microbial carbohydrases prepared from some varieties of A. niger were evaluated at the fifteenth meeting of the Committee, at which time a temporary ADI "not limited" was established (Annex 1, reference 26). A toxicological monograph was prepared (Annex 1, reference 27). An adequate 90-day study in rats was requested. Since the previous evaluation, additional data have become available on a number of carbohydrases, which are summarized and discussed in the following monograph. These enzymes were considered by the Committee to encompass the carbohydrases previously considered. The previously published monograph has been expanded and reproduced in its entirety below.

### AMYLOGLUCOSIDASES (E.C. 3.2.1.3)

### BIOLOGICAL DATA

#### Biochemical aspects

No information available.