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Toxicological evaluation of certain food additives and contaminants

Prepared by
THE 29th MEETING OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

IPCS · International Programme on Chemical Safety

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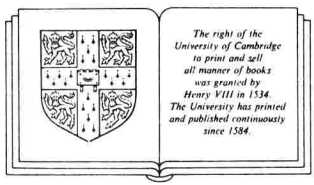

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PREFACE

The monographs contained in this volume were prepared by the twenty-ninth Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Geneva, Switzerland, 3-12 June 1985. These monographs summarize the safety data on selected food additives and contaminants reviewed 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 twenty-ninth report of JECFA has been published by the World Health Organization as WHO Technical Report Series, No. 733. 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 twenty-ninth 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. 34. 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 1985 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 of 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. Henceforth their publication by Cambridge University Press 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.

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 AND ENZYME IMMOBILIZING AGENTS

CARBOHYDRASE (α-AMYLASE) FROM BACILLUS LICHENFORMIS

EXPLANATION

Carbohydrase is an enzyme that catalyzes the hydrolysis of α-1,4-glycosidic linkages of starch. The enzyme preparation that is derived from B. lichenformis is added directly to the food to be processed and then it is removed from the final product by filtration. This preparation has not been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on genetic toxicity

Groups of 20 CD-1 male mice fed diets containing 0, 1.0, 2.0, or 4.0% of the carbohydrase preparation were used in a dominant-lethal study. The animals were fed the test compound for 5 days. These males were then mated 1-to-1 at weekly intervals with 5 different batches of 20 females each. At 14 days, after evidence of copulation, females were sacrificed and the uterine contents examined for implantations, viable embryos, and early and late embryonic deaths. Some weight loss occurred in both the high- and mid-dose males. One total-litter loss occurred in each of the high- and mid-dose groups at the second pairing and in the low-dose group at the third pairing, but the incidence of these losses was too low to be considered treatment-related and there were no other compound-related effects (Palmer & Lowell, 1973a).

A dominant-lethal study was carried out using groups of 20 male CD rats given a diet containing 0, 1, 2, or 4% of the carbohydrase preparation for 5 days. The males were then mated on a 1-to-1 basis for 7 days with untreated CD females. A new batch of females were mated with treated males every 7 days for 6 consecutive weeks. Pregnant females were sacrificed on about day 14 of pregnancy and ovaries and uteri were examined for corpora lutea, implantations, viable embryos, and early and late embryonic deaths. During the 5-day treatment period weight loss occurred in the high-dose males, and body-weight gains were reduced at the mid-dose level. There was no significant effect of treatment on mating performance, pregnancy rate, pre- or post-implantation loss, or overall viable litter size. Although 2 females in the high-dose group suffered total-litter losses, the overall incidence did not suggest a treatment-related effect (Palmer & Lowell, 1973b).

Special study on teratology

Groups of 20 mated female CD rats were fed diets containing 0, 1, 2, or 4% of the carbohydrase preparation from days 6 through 15 of pregnancy. The animals were sacrificed on day 20 of pregnancy and the uterine contents examined for the number of corpora lutea, number of viable foetuses, number of resorption sites, litter weight, and foetal abnormalities. One-third of the foetuses were examined by the Wilson technique for visceral abnormalities and two-thirds were processed for examination of skeletal abnormalities. During the first 4 days of dietary administration of the test compound there was reduced dietary consumption and reduced weight gain at all dose-levels (including weight loss at the high dose). No compound-related effects on foetal or embryological development were reported, although there was a non-significant increase in foetal abnormalities at the high dose (Palmer & Lowell, 1972).

Acute toxicity

Species	Route	LD_{50} (mg/kg b.w.)	Reference
Mouse	oral (gavage)	20,000	Novo, 1973a
Rat (male)	oral (gavage)	20,500	Novo, 1973b
(female)		16,500	

Short-term studies

Rats

Groups of 5 male and 5 female Wistar rats were fed diets containing 0, 0.5, or 2.5% of the carbohydrase preparation in the diet for 4 weeks. Except for a small but statistically-significant increase in absolute and relative kidney weights in the high-dose males, and some fluctuation in weight gain during the initial part of the study, there were no compound-related changes. Gross pathology, clinical chemistry, and feed efficiency were comparable between groups (Novo, 1972).

Groups of 15 male and 15 female CFY-strain rats were fed diets containing 0, 1, 2, and 4% of the carbohydrase preparation in the diet for 13 weeks. Thinning of the hair, mainly on the scapular region, was noted in 7 of the high-dose female rats from week 9 onward. There was reduced feed intake in mid-dose females and high-dose males and females, and decreased weight gain in high-dose animals of both sexes. Organ to body-weight ratios for several organs from the high-dose animals differed significantly from control values; however, many of these differences were likely to have arisen because of reduced body-weight gain. When compared to brain weights, only reduced liver weights in high-dose males were found to be significantly differently from controls. Increased adrenal weights in high-dose females were considered to be within the normal range of biological variability. Enlargement of the caecum was noted in mid-dose males and in both sexes at the high dose. No compound-related changes were reported with regard to survival, urinalysis, haematology, clinical chemistry, or microscopic pathology (Rivett et al., 1973a).

Dogs

Groups of 3 male and 3 female Beagle dogs were given diets containing 0, 1, 2, or 4% carbohydrase preparation for 13 weeks. Reduced mean body-weight gain was observed in high-dose animals of both sexes. Food and water consumption were reduced in high-dose animals of both sexes and in mid-dose females. No compound-related changes were observed with respect to haematology (1 high-dose female had platelet counts greater than the normal range), clinical chemistry, urinalysis, or gross and microscopic pathology. Differences between control and high-dose animals with respect to organ-weight ratios were ascribed to reduced growth of the high-dose animals (Rivett et al., 1973b).

Long-term studies

No information available.

Observations in man

No information available.

Comments

The carbohydrase preparation showed no significant toxicological effects in short-term feeding studies in rats at levels of up to 4% of the diet (40 mg/kg of feed) or in dogs at levels of up to 2% (20 mg/kg of feed). No teratogenic effects were noted in a study in rats. The preparation was also inactive in dominant-lethal tests in rats and mice.

EVALUATION

Level causing no toxicological effect

The no-effect level in a short-term study in dogs was 2% of the diet, equal to 450 mg/kg b.w.

Estimate of acceptable daily intake for man

ADI "not specified".

REFERENCES

Novo (1972). Four week oral toxicicity study of Novo alkaline amylase in rats. Unpublished study by Novo Industria A/S. Submitted to WHO by Novo Industria A/S.

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Rivett, K.F., Sortwell, R.J., Newman, A.J., & Street, A.E. (1973b). Novo NAA toxicity studies in beagle dogs. Unpublished report of the Huntingdon Research Centre. Submitted to WHO by Novo Industria A/S.

GLUCOSE ISOMERASE (IMMOBILIZED) FROM ACTINOPLANES MISSOURIENSIS

EXPLANATION

This enzyme preparation has not been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special study on reproduction

Rats

Groups of 20 male and 20 female Sprague-Dawley rats were fed diets containing 0 or 1% whole, non-viable Actinoplanes missouriensis in the diet. After 60 days on test, males and females were mated on a 1-to-1 basis. The males were sacrificed after 90 days on test for organ-weight analysis and gross and microscopic pathology evaluation. The females were allowed to litter and nurse their young until weaning at 21 days. Body weights tended to be lower in male rats given the test compound but did not differ significantly from controls. Right gonad weights tended to be slightly higher in treated males and females. No compound-related changes were observed in haematology, urinalysis, clinical chemistry, or gross or microscopic pathology, nor were there any effects on reproductive performance of the offspring (Tisdel & Harris, 1974a).

Acute toxicity

Species	Route	LD_{50} (mg/kg b.w.)	Reference
Rat (male)	Oral (dietary)	40,000	Regel, 1973
Mouse (male)	i.v.	1,250	Regel, 1973
Mouse (male)	i.v.	875-1,250	Regel, 1973
Rabbit	s.c.	1,250	Regel, 1973

Short-term study

Dogs

Groups of 2 male and 2 female beagle dogs were fed for 90 days diets containing 0 or 1% whole, non-viable cells of Actinoplanes missouriensis. Body-weight gains and food consumption were less for the test dogs than controls, but remained in the normal range for dogs of their age during the course of the study. Results of haematology and urinalysis were normal for all animals and no compound-related effects on organ weights or gross and microscopic pathology were reported (Tisdel & Harris, 1974b).

Long-term studies

No information available.

Observations in man

No information available.

Comments

A well-conducted short-term study in rats, which included a 1-generation reproduction study, showed no significant toxicological effects. A short-term study in dogs provides additional information on the lack of toxicity of the preparation.

The studies on the non-immobilized enzyme were considered by the Committee to be appropriate for evaluating the immobilized form because the use of gelatin as an entrapping agent does not present a toxicological problem. The release of free glutaraldehyde from the