ENZYMES DERIVED FROM ASPERGILLIS 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.

Toxicological studies

Special studies on aflatoxin-related effects

Ducklings

Four groups of 5 ducklings received in their diet 0, 1, 5, or 10% enzyme preparation for 29 days. Growth, feed consumption, survival, behaviour, and mean liver weights were comparable, in all groups. No gross or histopathological lesions of the liver were seen (FDRL, 1963a).

Four groups of 5 ducklings received in their diet 0, 1, 5, or 10% enzyme preparation for 29 days. Growth, feed consumption, survival, behaviour, and development were comparable in all groups. No gross liver lesions were seen at autopsy and mean liver weights of treated animals were similar to those of controls. Histopathology of the livers was normal. No toxic elements were noted (FDRL, 1963b).

Acute toxicity[1]

Species	Route	LD_{50} (mg/kg b.w.)	Reference
Mouse	oral	> 3,200	Hunt & Garvin, 1963
		> 4,000	Hunt & Garvin, 1971
		> 3,200	Willard & Garvin, 1968
		> 4,000	Garvin et al., 1966
Rat	oral	10,000	Gray, 1960
		31,600	Kay & Calendra, 1962
		> 3,200	Willard & Garvin, 1968
		> 4,000	Garvin et al., 1966
		12,500 - 20,000	Kapiszka & Hartnage, 1978
Rabbit	oral	> 4,000	Garvin et al., 1966
Dog	oral	> 4,000	Garvin et al., 1966

[1] These data were obtained with several different commercial enzyme preparations.

Short-term studies

Rats

Three groups of 10 male rats received 0, 0.5, or 5% enzyme preparation in their diets for 30 days. No adverse effects related to treatment were observed regarding growth, appearance, behaviour, survival, food consumption, haematology, organ weights, or gross pathology (Garvin et al., 1966).

Two groups of 10 male and 10 female rats received either 0 or 5% enzyme preparation in their diets daily for 91 days. No differences from controls were observed regarding appearance, behaviour, survival, weight gain, haematology, organ weights, or gross pathology (Garvin & Merubia, 1959).

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 between test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 of the study in both test and control animals. 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

Several short-term feeding studies in rats on amyloglucosidase preparations from A. niger have been performed. One study, in which the preparation was fed at up to 10% of the diet, was considered to be acceptable by current standards. No compound-related effects were observed in this study or in duckling tests that were performed to investigate potential aflatoxin-related effects.

The evaluations by the Committee of the carbohydrates and the protease from A. niger are summarized at the end of this section.

REFERENCES

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ß-GLUCANASE (E.C. 3.2.1.6)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

(The TOS of the enzyme preparation used for toxicity studies was 49%).

Special Studies on mutagenicity

The enzyme preparation was tested for mutagenic activity using 5 strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538 both with and without metabolic activation (S-9 fraction). The preparation was not mutagenic or toxic at concentrations up to 40 mg/ml (McConville, 1980).

A cytogenic bone marrow study was performed using adult male Chinese hamsters. Groups of adult male hamsters received up to 5000 mg/kg b.w./day of the enzyme preparation for 5 consecutive days. Treatment did not result in an increased frequency of chromosomal aberrations in bone marrow (McGregor & Willins, 1981).

Acute toxicity

Species	Route	Sex	LD_{50} (ml/kg b.w.)	Reference
Mouse (NMRI)	oral	M & F	30	Novo, 1978a
Rat (Wistar)	oral	-	28.1	Novo, 1978b

Short-term studies

Rats

Three groups, each containing 5 male and 5 female Wistar/Mol SPF rats, were dosed orally by garage once a day for 14 days with enzyme preparation at dose levels equivalent to 2.5, 5.0, or 10 ml/kg b.w. No clinical changes were observed. Body-weight gains of test and control animals were similar. At termination of the study, measurements of organ weights showed no compound-related effects (Novo, 1978c).

In another study, 4 groups, each containing 15 male and 15 female Wistar/Mol SPF rats, were dosed by gavage once a day for 90 days with enzyme preparation at dose levels equivalent to 0, 2.5, 5.0, or 10 ml/kg b.w. Deaths, primarily in the high-dose group, appeared to be related to injury during dosing. No clinical signs were observed in the other test animals. Male rats in the high-dose group showed decreased weight gain and marked decrease in food intake. Haematology studies showed increased platelet counts and decreased clotting times

in the high-dose group at week 6, but this effect was not apparent at week 12. No other effects were reported. Clinical chemistry and urinalysis values at weeks 6 and 12 were within the normal range. At termination of the study, organ weight analysis showed a marked increase in relative weights of the spleen and testes of the males in the high-dose group. Gross and histopathological examination of the principal organs and tissues showed no compound-related effects (Perry et al., 1979).

Dogs

Three groups, each containing one male and one female beagle dog, received single doses of 5, 10, or 15 ml/kg b.w. of the enzyme preparation over a 4-day period. Following a 7-day observation period the dogs were sacrificed and subjected to macroscopic post-mortem examination. No compound-related effects were observed, with the exception of vomiting during the first 4 days of the study. In another study, dogs were administered consecutive doses of 15 ml/kg b.w./day for 9 days, and 10 ml/kg b.w./day for 5 days. No deaths occurred during the course of the study. The only clinical sign noted was excessive salivation and emesis shortly after dosing. Body weights, electrocardiograms, haematological parameters, blood serum chemistry, organ weights, gross pathology, and histopathology showed no compound-related effects (Osborne et al., 1978).

In another study, three groups, each containing 3 male and 3 female beagle dogs, were dosed with the enzyme preparation by gavage once a day, seven days a week, for 13 weeks, at dose levels equivalent to 2, 5, or g ml/kg b.w./day. Two dogs in the high-dose group died during the course of the study, which the authors concluded was due to respiratory distress as a result of foreign material in the lungs. Vomiting was reported after dosing in the high-dose group. Haematological parameters at weeks 6 and 12 were within normal limits, with the exception of a significant increase in WBC count, specifically in the group mean neutrophil counts, in the high-dose group. Clinical chemistry values were within the normal range at weeks 8 and 12, with the exception of slight increases in blood glucose and cholesterol in the high-dose group. Urinalysis showed no compound-related effects. At termination of the study, organ-weight analyses and gross and histopathological examination of the principal organs and tissues showed no compound-related effects (Greenough et al., 1980).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

This enzyme preparation was not genotoxic in microbial or in mammalian test systems. Short-term studies in rats and dogs resulted in no observed compound-related effects at levels up to 5 ml/kg b.w./day of enzyme preparation.

The evaluations by the Committee of the carbohydrases and the protease from A. niger are summarized at the end of this section.

REFERENCES

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HEMI-CELLULASE

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on mutagenicity

The enzyme preparation was tested for mutagenic activity using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 both with and without metabolic activation (S-9 fraction). The test substance was not mutagenic or toxic at concentrations up to 5 mg/plate (Clausen & Kaufman, 1983).

In an in vitro cytogenetic test using CHO-K1 cells, both with and without metabolic activation (S-9 fraction), the enzyme preparation at test levels up to 2.5 mg (dry wt)/ml did not induce chromosomal aberrations (Skovbro, 1984).

Acute toxicity

No information available.

Short-term studies

Rats

Four groups, each containing 5 male and 5 female Wistar MOL/W rats, were dosed by garage once a day for 90 days with the enzyme preparation at doses equivalent to 0, 100, 333, or 1000 mg/kg b.w./day. No significant clinical changes were observed. Body-weight gain and food intake were similar among test and control animals. Haematologic and clinical chemistry measurements at termination of the study were within normal ranges. Post-mortem examinations, measurements of organ weights, and histopathology showed no compound-related effects. Slight increases in kidney and adrenal weights in the mid-dose group were not associated with histopathological effects, and did not show a dose response (Kallesen, 1982).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

This enzyme preparation was not genotoxic in microbial or in mammalian test systems. In a limited 90-day study in rats, no effects were observed at the highest dose administered (1 g/kg b.w./day). This enzyme preparation contained high levels of pectinase. The pectinase enzyme preparation summarized below may be identical to this hemi-cellulase preparation, which provides added assurance of the safety of this preparation.

The evaluations by the Committee of the carbohydrases and the protease from A. niger are summarized at the end of this section.

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PECTINASE (E.C. 3.1.1.11; 3.2.1.15; 4.2.2.10)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies (The TOS of the commercial preparation is approximately 5%).

Acute toxicity

Species	Route	LD_{50} (ml/kg b.w.)	Reference
Rat	oral	18.8-22.1	Porter & Hartnagel, 1979

Short-term studies

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 of the enzyme preparation/kg b.w./day), for 90 to 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 test and control groups. Differential blood counts at weeks 4 and 8 of the study were within the normal range in test and control animals. At the end of the study serum clinical chemistry analyses, 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

In a short-term study in rats, no adverse effects were observed at dietary levels of the enzyme preparation up to the equivalent of 7 mg/kg b.w./day. This enzyme preparation may be identical to the hemi-cellulase preparation summarized above. The hemi-cellulase enzyme preparation summarized above also contained high levels of pectinase, which provides added assurance of the safety of this preparation.

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PROTEASE

No information available.

GENERAL COMMENTS ON ENZYMES FROM A. NIGER

Aspergillus niger is a contaminant of food. Although there may be posible strain differences in A. niger, and different cultural conditions might be used to prepare the various enzymes, the available toxicity data, which consist primarily of short-term feeding studies in rats and some studies in dogs, show that all the enzyme preparations tested were of a very low order of toxicity. The enzyme preparations tested were non-mutagenic in bacterial and mammalian cell systems. Studies on some strains of A. niger used to prepare carbohydrases showed no aflatoxin or related substance production. These studies provide the basis for evaluating the safety of enzyme preparations derived from A. niger. It was also noted that the enzyme preparations tested exhibit a number of enzyme activities, in addition to the major enzyme activity. Thus, there may be considerable overlap of the enzyme activities of the different enzyme preparations so that safety data from each preparation provides additional assurance of safety for the whole group of enzymes.

Since the enzyme preparations tested were of different activities and forms, and most of the organic materials in the preparations are not the enzyme per se, the numerical ADI is expressed in terms of total organic solids (TOS) (see introduction to enzyme preparations section).

EVALUATION

Level causing no toxicological effect

All enzyme preparations tested showed no-observed-effect levels greater than 100 mg TOS/kg b.w./day in 90-day studies in rats.

Estimate of acceptable daily intake

0-1 mg TOS/kg b.w. for each of the enzyme preparations.

See Also:

Toxicological Abbreviations