Commission of the European Communities

food
science and techniques

# REPORTS OF THE SCIENTIFIC COMMITTEE FOR FOOD

## (Twenty-seventh series)

Report
EUR 14181 EN

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**(Twenty-seventh series)**

Foods intended for weight control diets
*(Opinion expressed on 19 October 1990)*

Guidelines for the presentation of data on food enzymes
*(Opinion expressed on 11 April 1991)*

Recommendation on cyclamates
*(Opinion expressed on 21 June 1991)*

Report on the risks of hypervitaminosis A
*(Opinion expressed on 21 June 1991)*

Directorate-General
Internal Market and Industrial Affairs

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*For their valuable and kind assistance to the study on "Food intended for weight control diets", the Scientific Committee for Food wishes to thank:*

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# Report of the Scientific Committee for Food on foods intended for weight control diets

*(Opinion expressed 19 October 1990)*

## Terms of reference

To advise on the use and on the essential requirements of products intended for use in weight control diets.

## Documents consulted

In drafting its opinion the Committee considered the work carried out on the same subject by other groups, notably Codex Alimentarius. The Committee examined many published papers in the area of obesity and slimming and also received valuable information from the association of Dietetic Foods Industry of the EC (IDACE).

## General considerations

Obesity is defined as an excessive accumulation of fat in the body leading to a body weight outside specified normal limits. In quantitative terms obesity is deemed to exist where body weight is 20 % above the upper limit of normal range (Bray, 1979; DHSS-MRC, 1976) for a given height. An alternative approach is to express the ratio of weight (kg) to the square of height (m) to yield the Quetelet or Body Mass Index (BMI) resulting in the following ranges which correspond to the generally accepted recommendations of the Fogarty Centre Conference on Obesity (Bray, 1979).

| | **BMI *** | |
|---|---|---|
| | **Men** | **Women** |
| Underweight | <20 | <18 |
| Desirable range | >20<25 | >18<23 |
| Overweight | >25<30 | >23<28 |
| Obese | >30 | >28 |

* [BMI $= \frac{\text{wt (kg)}}{\text{ht}^2 \text{ (m)}}$ ]

The relationship between adverse health effects and body weight is generally regarded as being J-shaped although this becomes less obvious when age is considered. In general the greatest risks in terms of mortality are associated with obesity in the age range 20-29 years (Royal College of Physicians, 1983). The disorders most frequently associated with obesity include hypertension, hypercholesterolaemia, diabetes (non-insulin dependent) and gall bladder disease (Hautvast & Deurenberg, 1987). Several of the conditions which are found more frequently among the obese, are themselves also associated with increased risk of coronary heart disease. Nonetheless the relationship between obesity and coronary heart disease remains complex. Thus the recent data of Manson *et al.* (1990) shows that the greater the BMI the greater the relative risk of fatal and non-fatal myocardial infarction in women, this occurs to only a moderate extent among the normotensive, normocholesterolaemic, non-smoking or the non-diabetic sub-groups The relationship between body weight and coronary heart disease may become clearer in the future as recent work develops on the importance of body fat distribution as a risk factor. A high waist:hip ratio, indicative of abdominal obesity, has been indicated by several longitudinal studies to be an important risk factor for heart disease (Larsson *et al.*1984). Thus the available evidence clearly indicates that obesity increases the risk of cardiovascular and other diseases but account must be taken of age (Andres *et al.*,1985), of body fat distribution. (Larsson *et al.*,1984) and of the relatively small effect of obesity on heart disease independant of smoking, blood pressure, blood cholesterol and adult onset diabetes (Manson *et al.*,1990).

The treatment of obesity has long been regarded as an extremely difficult area, leading to a plethora of diets promising beauty, virility and longevity at little personal costs, other than perhaps monetary. In considering the treatment of obesity, the Council of Scientific Affairs of the American Medical Association (1988) concluded that "Despite a multitude of programs designed to treat obesity, success in treatment is often difficult to achieve. If a cure for obesity is defined as reduction to desired weight for five years, it is more likely that most people will be cured of most forms of cancer than obesity". The high relapse rate in the treatment of obesity must remain an important factor in evaluating the suitability of a proposed treatment. Equally the variable health risks associated with obesity must remain an important consideration. A treatment with a moderate attendant risk is not acceptable in the treatment of individuals in the overweight or even mildly obese category, particularly given the high relapse rate. As the health risks associated with obesity increase with the degree of obesity, the acceptance of the risk associated with therapy also increases, notwithstanding the high relapse rate. This is reflected in the various guidelines for the treatment of obesity. The overweight category (BMI: male >25<30, female >23<28) is relatively low risk and can be treated by diet and by exercise. With BMI averaging between 30 and 40, more vigorous dietary therapy is required. Above a BMI of 40, invasive procedures involving small intestinal resection and jaw wiring are often used. Diet therapy is nonetheless central to the management of even the most massively obese.

In the US, the second National Health and Nutrition Examination Survey (NHANES II, National Centre for Health Statistics, 1983) showed that about 34 million American women aged 20-74 years were overweight (BMI >27.5) of which some 12.5 million were obese (BMI >31.7). These figures correspond to about 25 % of the population overweight with some 8% obese.

European data suggest that the incidence of obesity (>120% of ideal weight or BMI >30) is considerably less than in the US with values from as low as 2% for young Dutch adults, to 5-12% for a nationally representative UK sample to 23% in the Mediterranean EC countries (James *et al.*,1988). All of these data contrast with the very large numbers of individuals who

regard themselves as fat, some 82% of women of normal weight and some 45% of women underweight (Wooley, 1988) which is reflected in the very large numbers of people, male and female, who regularly diet.

Given the huge demand for successful diet it is not surprising that many faddish diets have come and gone and indeed come back again. Equally, it is not surprising that industry provides energy reduced versions of foods using sugar and fat substitutes and in recent years provides a comprehensive diet package which will ensure that an upper limit of energy intake is achieved. It is against this background that the Scientific Committee for Food examined the essential requirements of foods for particular nutritional uses intended for use in weight control diets. The Committee has attempted to strike a cautious balance between the real risks of obesity, the risks of the treatment of obesity and the degree of long term efficacy of treatment.

## Categories of products intended for use in weight control diets covered in this report

Products for use in weight control diets can be broadly classified into two main groups. The first group of products set out to provide all of the daily nutritional requirements with the exception of energy. The degree of energy deficiency allows this groups to be further subdivided into those which provide a substantial deficit in energy requirements (very low calorie diets) and those which provide a modest deficit in energy (low calorie diets). The second group of products are not intended to provide all of the daily nutritional requirements. They can also be divided into two categories, those products intended to replace one meal, usually the main meal, and those intended to complement a self-selected calorie controlled diet comprised of normal foods.

Each of these four categories will now be reviewed in greater detail.

## Very low calorie diets

Products very low in calories [less than 800 kcal (3360 kJ) per day and frequently less than 400 kcal (1680 kJ) per day] have come into wide circulation in recent years as slimming diets. The earlier products, often referred to as liquid protein diets, were associated with a number of fatalities largely due to the low biological value of the protein used. Since then, the quality of the protein used in such products has been considerably improved. One of the difficulties in evaluating the safety of very low calorie diets is the lack of a properly structured study of their widespread clinically unsupervised use by the moderately overweight. Reference to the extensive use of very low calorie diet as evidence of their safety when widely used by the general public is not an acceptable substitute for properly designed clinical studies. Equally, there is inadequate data on the long term efficacy of very low calorie diets when used outside the realm of clinical investigation units. In a comparison of the treatment of obesity with very low calorie diets alone, by behavioural therapy alone or by a combination treatment, Wadden *et al.*,(1988) found a three year relapse rate of 74-87% across treatments with no differences in weight loss between treatments. The data available from industry would also indicate that only 24-36% of the users of very low calorie diets follow the label instructions to consult with a doctor before starting to take such diets and that some 25%

of users of very low calorie diets ignore label instructions not to pursue the diet for longer than three weeks (cited in DHSS, 1987). A central and unresolved problem surrounding the use of very low calorie diets is the possibility that a disproportionate part of the weight loss is derived from lean tissue in some individuals even after a substantial period of adaptation (Atkinson, 1989) and that this loss is greatest among less obese individuals (Forbes, 1987). The Committee also noted recent data which showed that in a study comparing very low calorie diets (500 kcal or 2100 kJ per day) and a balanced deficit diet (1200 kcal or 5040 kJ per day), the rate of weight loss did not differ, (Foster *et al.*,1990). The same group also compared 48 weeks use of a self selected balanced deficit diet (1200 kcal/d. or 5040 kJ/d.) against the same diet for 26 weeks commencing after a preliminary 22 weeks on a very low calorie diet (420 kcal/d.; 1757 kJ/d.). At the end of the 48 weeks study, no significant advantage in terms of weight loss was evident in the group partially treated with a very low calorie diet (Wadden *et al.*,1990). Given the continuing concern over the irresponsible uses of very low calorie diets (Wadden *et al.*,1990), the Committee were unanimous in their view that very low calorie diets should not be freely available to the general public for weight reduction. The Committee feels that given the balance of risk and benefit, for the general population, access to commercially prepared products should be limited to those which provide a minimum of 800 kcal or 3360 kJ per day. Very low calorie diets, providing less than 800 kcal or 3360 kJ per day should only be available for the properly supervised treatment of severe obesity. Accordingly, very low calorie diets should only be available on prescription and sold exclusively through pharmacies. Therefore they will not be considered in this report which will focus only on the other three categories.

## Category 1. Low calorie diets (800-1200 kcal or 3360-5040 kJ per day)

Weight reduction frequently requires quite long term adherence to low energy diets, even when a suitable body weight has been attained maintaining that weight will require re-introduction of an energy-reduced diet at various intervals. Although these diets are quite feasible using ordinary foodstuffs, the constraints of modern lifestyles often make commercially prepared, nutritionally complete, low energy diets attractive to many people. Industry has responded to this demand by providing a wide range of such products from full formula diets to diets comprising a variety of meals. Thus a minimum supply of protein, vitamins and minerals must be specified to ensure that energy is the only nutrient which is deficient. This category of products should provide energy in the range of 800-1200 kcal or 3360 - 5040 kJ per day.

## Category 2. Meal replacement products (275-400 kcal or 1155-1680 kJ per meal)

Many individuals on a low energy diet do not want to replace all their meals by commercially designed products. A demand exists for products to replace one meal per day, usually the main meal. Such products should therefore contain sufficient quantities of minerals, vitamins and protein to ensure that adequate intakes of these nutrients are achieved. Products intended to replace one meal per day should be clearly labelled so. Manufacturers should advise the consumer as to how other meals might be balanced to ensure adequate intake while maintaining overall energy intake in the range of 800-1200

kcal or 3360 - 5040 kJ per day. This category of products should provide energy in the range of 275-400 kcal or 1155-1680 kJ per meal.

### Category 3. Products intended to complement a calorie controlled diet which is freely selected from normal foods (125 kcal or 525 kJ per item)

A number of products are available on the market which are intended to help individuals follow a calorie controlled diet. These nutrient dense products are frequently in the form of bars, biscuits, snacks etc. They are intended to help individuals who are following their own diet to eat products which are low in energy but rich in protein, minerals, vitamins and in some instances, in fibre. These products should provide less than 125 kcal per unit or 525 kJ per unit.

## Compositional standards of products intended for use in weight control diets

### Energy

Weight loss requires that energy expenditure should be higher than intake. Energy expenditure at rest is proportional to lean body mass and is about 1 kcal or 4.2 kJ per minute for the average person and equivalent to about 1500 kcal or 6300 kJ per day. Thus most slimming diets aim to provide about 1000 kcal or 4200 kJ per day. The Committee accepted a deviation of 20% from this for products intended to replace all meals, giving an energy range of 800-1200 kcal or 3360-5040 kJ for such products. Single meal replacements have their energy range set at about one third of these values, ie. 275-400 kcal or 1155-1680 kJ per meal. The value of 125 kcal or 525 kJ per item for products intended to complement a freely selected low energy diet was chosen to ensure that such products would be clearly distinguishable from meal replacement products.

### Protein

Individuals following an energy reduced diet should ensure that protein intake is adequate to maintain nitrogen balance. The current adult safe protein allowance is 0.75 g/kg body weight per day (FAO/WHO/UNU 1985). This translates into a value of about 50 g/d for the average person of 70 kg body weight. Where products are intended to replace all the day's meals, protein should be provided at a level of 50 g per day which corresponds to about 20% of energy. This value of 50 g is a minimum for good quality protein i.e. 100% of the reference protein (FAO/WHO/UNU 1985). It may be increased if the biological value of the protein is lower, although the biological value should never be lower than 80 % of the reference protein. For such products, intended to replace all of the days meals, an upper limit of 50% of dietary energy from protein is set. This is partly to ensure that a reasonable balance of lipids and carbohydrate should be allowed in the products and also to ensure that the renal load of nitrogenous waste is not excessive.

Products intended to replace one meal per day should contain a minimum of at least a third of the daily requirement for protein i.e. 17 g, and of the same quality as described for the low calorie products. This minimum of 17 g of protein per meal ensures that should such products be improperly taken to replace all of the day's meals, adequate intakes of protein will be guaranteed. Again, an upper limit of 50% of dietary energy from protein for meal replacement products is set to ensure a good balance of carbohydrates and lipids.

In the case of nutrient dense products intended to be used as a supplement to energy reduced diets, the protein content should be higher than that proposed for other categories. This recommendation is based on the fact that such products are intended to have a high nutrient density to allow people following a self selected slimming diet to achieve a good intake of proteins, minerals and vitamins. Thus the Committee recommends that the protein should provide between 30 and 50% of the energy of such products giving a range per product of 10-15 grams. The proteins should be of the same quality as that defined for other products.

## Lipids

Generally speaking it was considered desirable by the Committee that the fat content should not exceed 30% of dietary energy for any of the product categories considered. An adequate intake of essential fatty acids should be maintained. A figure of 2-3 % of energy from essential fatty acids is generally considered to be sufficient to meet the needs of most individuals. Thus in products intended to provide all of the day's nutritional requirements, a minimum intake of 4.5 g per day of cis-cis linoleic acid should be provided. This is based on the normal average intake of 2,000 kcals or 8.4 MJ per day to provide 2.0 % of energy from linoleic acid. For products intended to replace one meal, the minimum level of linoleic acid should be 1.5 g per serving which could be provided by about 4 g of an appropriate vegetable oil rich in polyunsaturated fatty acids and which would remain within the constraints of 30% of energy from fat. In the case of products intended to complement a freely selected low-energy diet, the Committee did not feel that specific recommendations should be set.

## Carbohydrate and sugar alcohols

Having set limits on the level of proteins and lipids which may be included in the various product categories, the remaining energy can be provided by carbohydrates. The calorie content of sugar alcohols (polyols) which may be used in such products cannot be ignored and the Committee recommends that for calculation purposes, the sugar alcohols be considered to have an energy value of 2.4 kcal/g (10 kJ). Where the intake of polyols may exceed 20 g per day, this should be stated on the label which should also advise consumers that consumption of the product may have a laxative effect.

## Fibre

There is at present a controversy as to the best method for defining fibre. The definition depends on whether fibre is defined by its chemical composition (Englyst method) or by its physiological function in the large intestine (Southgate method). The chemical definition confines fibre to the non-starch polysaccharides (with or without lignin). The physiological definition confines fibre to that fraction of the dietary carbohydrates

(including lignin) which cannot be digested in the small intestine and which is available, in part at least, for fermentation by colonic anaerobes. The by-products of this fermentation may be absorbed across the colon and be available for subsequent metabolism. This definition of fibre includes certain fractions of starch which are resistant to hydrolysis by pancreatic amylase. These resistant starches may occur naturally in foods or may arise in cooking or during manufacture. Given the problem of properly defining fibre, the Committee proposes for the moment to use the physiological definition of fibre based on the Southgate method pending a detailed report on the matter by the Scientific Committee for Food.

The Committee recommends that to avoid constipation on the one hand and on the other the temptation to produce very bulky foods with excessive fibre levels, a minimum and maximum value should be set. Accordingly the Committee proposes a range of 10-30 g of dietary fibre per day for products intended to provide all of the day's meals. In the case of products intended to replace one meal, it may not be technologically possible to include fibre to the minimum level of 10 g/1000 kcal or 4200 kJ. Where this arises the products should carry a clear statement advising that the product does not contain adequate fibre and that fibre should be obtained from other sources. In the case of products intended to complement a self selected low energy diet, the Committee did not feel that specific recommendations for dietary fibre should be set.

## Minerals and vitamins

The Scientific Committee for Food is currently detailing recommended dietary allowances (RDA's) for use in the European Community. Thus while the guidelines in this report approximate the generally accepted values for RDA's currently in operation in the European Community, the Committee recommends that these be modified when the work of the Committee on RDA's has been completed. Thus the following minimum values are set for products intended to replace the normal diet.

**Units per 1000 kcal (4200 kJ)**

| | | | |
|---|---|---|---|
| Calcium | 800 mg | Vitamin A * | 800 µg |
| Magnesium | 300 mg | Vitamin D | 2.5 µg |
| Potassium | 1500 mg | Vitamin E | 10 mg |
| Sodium | 1500 mg | Vitamin C | 50 mg |
| Iron | 14 mg | Thiamin | 1.4 mg |
| Zinc | 14 mg | Riboflavin | 2.0 mg |
| Iodine | 140 µg | Niacin | 24 mg |
| Phosphorus | 800 mg | Vitamin $B_6$ | 2 mg |
| | | Folic acid | 100 µg |
| | | Vitamin $B_{12}$ | 1 µg |

* Retinol equivalent

For products intended to replace only one meal, one third of these requirements must be met per meal. In the case of products intended to complement a self selected low energy diet one fifth of requirements for Ca, Fe, vitamins A, D, E, thiamine, riboflavin and $B_6$ should be met per product. For each product category, the levels of vitamins A and D should not exceed twice the specified minimum requirement.

## Labelling and claims

The Committee recommends that each category of product be clearly labelled, so that consumers do not confuse one category for another. Other than those intended to supply all nutrients for the day, all products should clearly warn consumers to obtain the rest of their nutrients from ordinary foods. In the case of the products intended to replace all of the day's meals, the product label should advise that this type of diet should not be taken for more than three consecutive weeks as the sole source of nutrition without seeking medical advice.

In the case of products intended to replace all of the day's meals, the distribution of nutrients in each portion need not be specified provided that the macronutrients are reasonably diversified across the various components of the daily ration. However, each component of the product should clearly state that it is to be used as directed in conjunction with all other components of the product. Where products are free of lactose this may be stated on the label.

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# Annex

## Compositional standards of products intended for use in weight control diets

| | | Category 1<br>To provide all the daily nutrients in a low energy diet<br>*units per day* | Category 2<br>To replace one meal per day<br>*units per meal* | Category 3<br>To complement a self-selected low energy diet<br>*units per product* |
|---|---|---|---|---|
| Energy | kcal | 800-1200 | 275-400 | <125 |
| | kJ | 3360-5040 | 1155-1680 | <525 |
| Protein [a] (minimum) | (g) | 50 | 17 | 10 |
| *Cis-cis* linoleic acid | (g) | 4.5 | 1.5 | NS |
| Total fat (% energy) | | <30 | <30 | NS |
| Dietary fibre [b] | (g) | 10-30 | 10/1000 kcal [d] | NS |
| Calcium | (mg) | 800 | 270 | 160 |
| Magnesium | (mg) | 300 | 100 | NS |
| Potassium | (mg) | 1500 | 500 | NS |
| Sodium | (mg) | 1000 | 350 | NS |
| Iron | (mg) | 14 | 5 | 2.8 |
| Zinc | (mg) | 14 | 5 | NS |
| Iodine | (µg) | 140 | 47 | NS |
| Phosphorus | (mg) | 800 | 270 | NS |
| Vitamin A [c] | (µg) | 800 | 270 | 160 |
| Vitamin D | (µg) | 2.5 | 0.8 | 0.5 |
| Vitamin E | (mg) | 10 | 3 | 2 |
| Vitamin C | (mg) | 50 | 17 | NS |
| Thiamin | (mg) | 1.4 | 0.5 | 0.3 |
| Riboflavin | (mg) | 2.0 | 0.7 | 0.4 |
| Niacin | (mg) | 24 | 8 | NS |
| Vitamin $B_6$ | (mg) | 2.0 | 0.7 | 0.4 |
| Folic Acid | (µg) | 100 | 33 | NS |
| Vitamin $B_{12}$ | (µg) | 1.0 | 0.3 | NS |

(a) 100% of the Reference Protein (FAO/WHO/UNU 1985)
(b) As determined by the Southgate method
(c) Retinol Equivalents
(d) If technologically not possible, statement on labelling about necessity to obtain fibre from other sources
NS = non specified

# Guidelines for the presentation of data on food enzymes

*(opinion expressed 11 April 1991)*

## Introduction

These guidelines cover enzyme preparations intended for use in the preparation of food. Some enzyme preparations are used as processing aids and others as true food additives. Whilst this distinction may be of administrative importance, from a toxicological point of view it is not pertinent to distinguish between these two categories since, in both cases, the enzyme preparations may remain in the food.

These guidelines should be considered as setting out the minimum requirements for information to be supplied to the Scientific Committee for Food (SCF) in connection with any request for an evaluation of the safety in use of an enzyme preparation. If, for a specific enzyme preparation, some of the requirements are considered irrelevant, they may be omitted provided satisfactory supporting arguments are presented.

The **safety issues** involved in the use of enzyme preparations can roughly be split into the following categories:

1. **Toxicological properties of the enzyme preparation** (i.e. the active enzyme as such and any byproducts and contaminants). Care should be taken to ensure that toxic contaminants are not present in the enzyme preparation (e.g. mycotoxins and antibiotics in enzymes of microbial origin).

   In order to ensure a consistent and safe product there is a need:

   a) for process specifications including appropriate quality assurance checks;
      and
   b) to ensure that the characteristics of the source material or organism do not change with time.

2. **Quantity of enzyme consumed.** This in turn depends on the amount added to food, the concentration in the food when consumed, the number of different foods in which it may be used and the frequency with which these foods are consumed.

3. **Allergies and irritations caused by enzyme activity in the final product.** This is primarily considered to be an occupational health problem when workers are handling concentrated enzyme preparations. There are no confirmed cases of allergies induced in consumers through intake of enzyme treated food.

4. **Unintended reaction products in the food caused by enzymatic reactions in the final foodstuffs** (e.g. transformation of histidine to histamine). The possibility of any adverse health effects from this cause should be addressed in the submission.

5. **Safety of the source organism (microbials).** The use of pathogenic microorganisms is primarily of occupational concern as viable cells from the source organism should not be present in the final commercial enzyme, but as a general principle pathogenic microorganisms should not be used in the production of food enzymes

With regard to the **toxicological properties** of enzyme preparations, enzymes which are derived from edible parts of plants and animals are generally considered as posing no health problems. No special documentation for safety need be supplied provided that the potential consumption following normal use does not lead to an intake of any components which is larger than can be expected from normal consumption of the source as such, and provided that satisfactory chemical and microbiological specifications can be established.

For enzymes derived from microorganisms, it is very important to ensure that the source does not produce toxic compounds which can remain in the final product. It is important to carry out toxicological test programmes on all the individual strains used in the production of specific enzyme preparations since:

1. Different strains belonging to the same species can behave differently. For many microorganisms it is known that some of the strains in one species are harmless, while others belonging to the same species are toxic.
2. For some fungal genera, especially *Penicillium* and *Aspergillus*, there have been many misidentifications of fungal isolates. As a consequence of this, there is a risk of misclassification of fungal strains. For example in some cases it has been difficult to distinguish *A. oryzae* from *A. flavus* which has the ability to produce aflatoxin. As long as there is a risk of misidentification of microbial isolates, it is very important that the microorganism used is correctly identified and, in case of doubt, the identity should be verified by an independent, recognized laboratory.
3. The ability of a microorganism to produce toxins depends – qualitatively and quantitatively – on environmental factors such as the composition of fermentation media, pH, temperature and fermentation period. Therefore there is a risk that a microorganism which does not produce toxins under some conditions will turn out to be toxin-producing under other conditions.
4. The continuous selection processes applied to source microorganisms in order to maximize and optimize enzyme production may result in spontaneous mutations which give rise to the possibility of changing a non-toxic strain to a toxic strain.
5. There is a considerable potential to apply new techniques of genetic modification in the production of food enzymes. Along with the introduction of desirable traits, there is also the potential for introducing toxin production and therefore there is a need

explicitly to characterize and evaluate the genetic construct as to host, vector and insert (see section 2.4 under "Information to be supplied").

# Information to be supplied for the evaluation of an enzyme preparation to be used in foodstuffs

## Administrative data

Name of applicant, manufacturer(s) of enzyme and person responsible for the dossier.

## Technical data

### *1. Active components*

**1.1 The principal enzyme activities** are to be characterized by their systematic names and Enzyme Commission numbers.[1]

**1.2 The activity of the enzyme preparations** should be measured according to the reaction catalysed by individual enzymes and should usually be expressed in activity units per unit weight or volume of preparation as appropriate. In commercial practice the activity of the product is sometimes also given as the quantity of the enzyme preparation to be added to a given quantity of food in order to achieve the desired effect.

**1.3 A list of subsidiary enzymatic activities**, whether they perform a useful function or not.

### *2. Source materials*

If any specific source is likely to contain substances which may be harmful to health, the absence of such substances in the enzyme preparation shall be shown (see section 8.6).

**2.1 Animal sources.** The animal and the part of the animal used in the preparation must be identified. Animal tissues used for the preparation of enzymes must comply with meat inspection requirements and be handled in accordance with good hygienic practice.

**2.2 Plant sources.** The plant and the part of the plant used in the preparation must be identified.

**2.3 Microbial sources** used in the production of enzyme preparation can be native strains or variants of microorganisms, or be derived from native strains or variants by the process of selective serial culture or genetic modification. They must be discrete and stable strains or variants which are sufficiently well characterized according to well accepted identification keys, to enable them to be assigned unique identities as the

sources of the enzyme preparations which are the subject of individual specifications (see point 2 relating to enzymes derived from microorganisms in "Introduction").

The type cultures of the production microorganisms must be maintained under conditions which ensure the absence of strain drift and when prepared for use in the production of enzyme preparations they must be subjected to methods and culture conditions which ensure consistency and reproducibility from batch to batch. These procedures must ensure the absence of toxin production by the source organism and prevent the introduction of foreign microorganisms which could be the source of toxic materials and other undesirable substances in the final enzyme products.

2.4 **Genetically modified organisms.** The specification shall contain information about the host organism, the vector (plasmid) and the DNA-sequence incorporated in the vector or in the chromosome. Whether plant, animal or microorganism, the donor organism should also be identified.

It is important to have detailed knowledge of the genetic structures involved so that any undesirable interaction between the original genetic material of the host and the new genetic material to be inserted can be anticipated. Data on genetic structure such as information on presence of extra DNA (plasmids or foreign DNA incorporated in the host chromosome), specific genetic characteristics ("markers"), presence of dormant genes (which can be expressed by mutations), genetic stability (mutation rate and factors influencing the mutation rate, inter- and intramolecular recombinations, restriction barriers), gene transfer (mobilization/conjugation ability) and resistances (antibiotics, heavy metals) will assist in the prediction of undesirable effects on human health, animals, plants and ecological behaviour.

Exact knowledge of the identity and the biology of the vectors forms the basis for the evaluation of whether the introduction of the vector increases or reduces the safety level of the host microorganism. A vector should be characterized at the DNA level (size, restriction map and possibly full DNA sequence) and genetically with respect to genes found on the vector and which could be used as marker genes. A vector must be free of harmful sequences as well as non-conjugative and non-mobilizable.

The DNA sequence(s) to be inserted in the host organism has (have) to be fully characterized both at the molecular level and in terms of the number of inserted genes, type of regulation (promotor activity) and actual gene product(s). Whether the DNA sequence originates from a microorganism, plant or animal, the exact origin and pedigree of the genetic construct has to be given in order to enable a proper safety evaluation to be carried out.

Each recombinant product is to be evaluated on a case-by-case basis considering the host, the vector and the insert and taking into account that the potential hazard from the final product might be more than simply the sum of the single elements.

## 3. *Manufacturing process*

**3.1** Adequate information on the **method of manufacture**. For microbial sources information on fermentation media and conditions are considered essential. All components used must be of food grade quality.

**3.2** Adequate information on the **purification procedure** shall be given.

If changes occur in the manufacturing process or in the purification of the enzyme preparation it will be considered as new unless it can be demonstrated that the final product can be considered the same as that prepared by the original procedures.

## 4. *Carriers and other additives and ingredients*

**4.1** Informations on **carriers, diluents, excipients, supports and other additives and ingredients** (including processing aids) used in the production, distribution, and applications of enzyme preparations must be given. They must be substances that are acceptable for the relevant food-uses of the enzyme preparations concerned, or substances which are insoluble in food and removed from the food material after processing and before consumption.

**4.2** In the case of **immobilized enzyme preparations**, the carriers and immobilization agents used should be acceptable for the relevant use. When new materials are being considered, they should be tested to prove that no harmful residues will leak out into the food. Tests should be performed showing that any leakage of immobilization agents or enzymes is kept within acceptable limits as specified in the individual specifications.

**4.3** In order to distinguish the proportion of the enzyme preparation derived from the source material from that contributed by diluents and other additives and ingredients, individual specifications may require a statement of percentage Total Organic Solids (T.O.S.) which is defined as follows:

$$\% \text{ T.O.S.} = 100 - (A+W+D)$$

where A = % ash, W = % water and D = % diluents and/or other additives and ingredients.

The T.O.S. may be expressed as a ratio to the pure active ingredient (i.e.the enzyme content). Depending on the product in question the ratio may be very close to 1.

## 5. *Usage*

Information should be given on:

**5.1** Technological function of the enzyme.

**5.2** Types of foodstuffs in which the enzyme is intended to be used.

**5.3** Maximum amount of enzyme preparation to be used in each foodstuff.

### *6. Stability and fate in the food*

Information should be given on:

**6.1** Amount of enzyme preparation (i.e. active enzyme as well as other constituents) in the final food preparation.

**6.2** Main reaction products and possible reaction products not considered normal constituents of the diet, formed during the production and storage of enzyme treated food (see point 4 of the general safety issues in the Introduction).

**6.3** Possible effects on nutrients.

## General requirements and specifications

### *7. Hygiene*

**7.1** Enzyme preparations are to be produced in accordance with good food manufacturing practice. The stock cultures of microorganisms used as the sources of enzyme preparations should be periodically tested to ensure their purity (see section 2.3).

**7.2** Addition of the enzyme preparation to a foodstuff must not cause any increase in the total microbial count in the foodstuff.

### *8. Contaminants*

**8.1** **Heavy metals**: Preparations should not contain toxicologically significant amounts of heavy metals such as lead, cadmium, arsenic and mercury. The actual levels of heavy metals should be stated for each preparation.

**8.2** **Microbiological contaminants**

• No pathogenic micro-organisms (eg. *Salmonella*, *Shigella*, *Escherichia coli*, *Listeria*, *Campylobacter*, *Clostridium perfringens*) should be detectable using appropriate techniques.[2]

• Coliforms not more than 30 per gram as determined by a suitable method (eg. ISO 4832).[3]

• Total viable count not more than $10^2 - 10^4$ per gram as determined by a suitable method.[4]

**8.3** Tests shall be performed to ensure that viable cells from the microbial source organism are not present in the final product.

**8.4** Enzyme preparations may not contain any antibiotic activity as determined by a suitable method.[5]

**8.5** Enzyme preparations may not contain detectable amounts of toxins. When a given source is known to be able to produce toxins the absence of those toxins relevant to the organism shall be shown by a suitable method.

## Documentation for safety in use

### *9. Basic toxicological requirements*

**9.1** For enzymes derived from edible parts of animals or plants no toxicological tests are normally required. Where parts which are not generally considered as a normal part of the diet are used, some toxicological testing may be required unless other satisfactory documentation for safety in use is provided.

**9.2** For enzyme preparations derived from microorganisms the following tests are normally required:

(a) 90-day oral toxicity test in a rodent species;

(b) Two short-term tests:

1. a test for gene-mutations in bacteria,
2. a test for chromosomal aberrations (preferably *in vitro*).

The toxicological tests shall, where possible, be performed on a batch from the final purified fermentation product, before addition of carriers, diluents, etc. They should, as a general rule, be performed in accordance with established guidelines (EC/OECD; see also references [8] and [11]) although, because of the effects exerted at the cellular level by the proteinaceous nature and/or enzymatic activities of certain enzyme preparations, some modifications of the standard test protocols, particularly in the case of *in vitro* tests, may be necessary. Such deviations will be acceptable if accompanied by adequate supporting arguments.

The test system is designed to uncover unspecified toxic reactions and to reveal genotoxic effects. The combined information from the general specifications and this test battery make it possible to evaluate the product for the presence of both specific, well known toxins and unknown toxic compounds.

The toxicological report shall contain satisfactory documentation that the tests have been performed on the material which forms the basis of the commercial product as described in the technological dossier.

## *10. Exemptions from the basic toxicological requirements*

From a toxicological point of view it is important to perform a toxicological testing procedure on each specific enzyme preparation produced from a microbiological source.

**10.1** If, however, one enzyme preparation from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other enzymes from the same strain, the full testing battery may be waived for these enzymes. This will be decided on a case-by-case basis.

**10.2** If the microorganism used in the production

- has a long history of safety in food use, and
- belongs to a **species** about which it has been documented that no toxins are produced, and
- the actual **strain** used is of well documented origin,

the acceptance of an enzyme preparation from this organism without specific toxicological testing may be justified. In this case a correct and confirmed identification of the organism is of extra importance.

Presently the Committee can give only one example of such an organism which is *Saccharomyces cerevisiae*.

Enzyme preparations from such sources still have to comply with the general specifications.

**10.3** When a mutant strain is substituted for the original strain of microorganism used in the production of an enzyme preparation previously tested and approved, a modified, less comprehensive test procedure may be appropriate. Justification for such a reduced procedure must be provided on a case-by case basis.

**10.4** In connection with immobilized enzyme preparations, where immobilization techniques are evaluated and approved on the basis of adequate toxicity testing, they may be combined with previously evaluated and approved enzyme preparations without the need for additional toxicity testing on the combined product if analytical data are provided to indicate that the leakage of components of the combined product is within acceptable limits (see section 4.2).

**10.5** With the introduction of well specified, non-toxin-producing genetically engineered source organisms for the production of food enzyme preparations, it may in future be possible to produce enzymes of very high purity and specificity. For products where it is possible to demonstrate such high purity and specificity, the full toxicity testing may not be needed.

Notwithstanding the circumstances listed above where testing procedures may be acceptable, there may be circumstances where additional testing over and above the basic requirements is necessary to resolve questions that arise in any of the basic studies.

## Evaluation of safety in use

On the basis of technological and toxicological data submitted, the Committee will ascertain the safety in use of the enzyme preparation. This may be done either by defining the acceptable conditions of use or, when appropriate, by allocating an acceptable daily intake for a specified enzyme preparation based on the no-observed-effect-level in the sub-chronic rodent study with the application of a suitable safety factor.

The evaluation will be confined to the product described in the submission and cannot automatically be considered to cover other preparations of the same enzyme prepared from other sources or by other processes.

# Bibliography

During the course of formulating these guidelines the Committee had access to and considered the following documents and publications:

1. Enzyme Nomenclature, Recommendations (1984) of the Nomenclature Committee of the International Union of Biochemistry, Academic Press 1984.

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9. Principles for the safety assessment of food additives and contaminants in food (1987). Environmental Health Criteria 70, WHO.

10. Starterculturen und Enzyme für Lebensmitteltechnik. Mitteilung XI der Senatskommission zur Prüfung von Lebensmittelzusatz- und -inhaltsstoffen. Deutsche Forschungsgemeinschaft, VCH Verlagsgesellschaft, 1987.

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12. Evaluation of certain food additives and contaminants. 1990. 35th JECFA report. Technical Report Series 789. WHO, Geneva.

13. Thoughts on the industrial use of enzyme preparations made from genetically modified microorganisms. Report presented by France to Council of Europe. P-SG (90) 44, 21 December 1990 (restricted).

# Recommendation on cyclamates

*(Opinion expressed on 21 June 1991)*

1. Following the position recently taken on cyclamate by the UK's Committee on Toxicity in the light of new data, the Commission of the European Communities has asked the Scientific Committee for Food to review the data available for cyclamic acid and its sodium salt to see whether there is any basis for changing its existing opinion.

2. The Committee last reviewed cyclamate in 1988 (Reports of the Scientific Committee for Food, Twenty-first Series, 1989, EUR 11617). At that time it confirmed its earlier decision, reached in 1984, to allocate a group temporary ADI of 0-11 mg/kg bw to cyclamic acid and its sodium and calcium salts. The UK Committee on Toxicity has allocated a temporary ADI of 0-1.5 mg/kg bw to this sweetener.

3. In reviewing the data, the Committee noted that itself and the Committee on Toxicity agreed on the basic toxicological finding on which they based their ADI's, namely the testicular toxicity of cyclohexylamine (the gut floral metabolite of cyclamate) and its no-observed-effect-level in the rat of 100 mg/kg bw/day. There is also agreement on the degree to which cyclamate is absorbed; that the extent of conversion of cyclamate to cyclohexylamine varies in different individuals from 0 to 100% of the available cyclamate; and that any safety evaluation should take into account especially sensitive sub-groups in the population.

4. The difference in the position taken by the COT to that taken previously by this Committee stemmed from the different choice made concerning the extent of conversion of cyclamate to cyclohexylamine in the human population and in the choice of safety factor to be applied to the NOEL in deriving the ADI.

5. Whilst acknowledging the existence of new data, the Scientific Committee for Food is of the opinion that the new data do not introduce any significantly novel aspect into the consideration of cyclamate. The choice of the appropriate conversion factor in combination with the safety factor remains one of judgement. Such a judgement must take into account the fact that different individuals in the population convert cyclamate to different extents and that the ability of any one individual to convert varies with time.

The Committee is of the view that its previously assumed conversion rate of 30% of the available cyclamate (equivalent to 19% of the total ingested) is sufficiently conservative to provide adequate assurance of safety for the overwhelming majority of the population and re-affirms its view that the safety factor of 100 applied in determining ADI covers lifetime exposure and therefore provides sufficient assurance of safety to encompass those few individuals who may be converting to a greater extent at any one time.*

6. In conclusion, the Committee confirms its existing group temporary ADI of 0-11 mg/kg bw for cyclamates. Before a full ADI can be established there is a need for more information concerning the degree to which cyclamate is converted to cyclohexylamine. The Committee requests that studies be carried out to define with greater precision the range of extent of conversion in humans with respect to variation with time in any individual. Such studies should pay particular attention to the high converters in the population. *In vitro* studies on testicular tissues should also be carried out to determine the sensitivity of man relative to species for which data already exist. The results of these studies should be available to the Committee by the end of 1992.

* One member (Dr J Steadman ) disagreed with this view.

# Report on the risks of hypervitaminosis A

*(Opinion expressed on 21 June 1991)*

1. The Committee has examined the available experimental and epidemiological data which would permit an evaluation of the risks from hypervitaminosis A. These risks are related in the Committee's opinion essentially to a teratogenic effect in women during the first two months of pregnancy.

2. A teratogenic risk appears possible, albeit very low, from a chronic intake of 20,000 IU vitamin A per day and seems likely at doses higher than 50,000 IU per day. The effects of a large dose ingested haphazardly are more difficult to estimate. In the present state of knowledge it is possible to hypothesise that the teratogenic effect of vitamin A is due to an excessive production of retinoic acid which then acts abnormally on those nuclear receptors which affect the expression of genes involved in cellular differentiation once the binding capacities of retinoic acid to the Cellular Retinoic Acid Binding Protein have been exceeded. Under these conditions it is to be feared that the effects of a single, large dose of vitamin A would be more important than the same dose divided into regular daily ingestions.

3. The Committee noted the analytical data, collected in the different Member States, on the vitamin A content of the livers of animals raised for human consumption. It also noted that levels exceeding 100,000 IU/100 g liver occurred rather frequently and that in certain cases even 400,000 IU/100 g liver could be reached.

4. The Committee is of the opinion that such levels constitute a risk for the health of the public and that immediate measures should be taken regarding animal husbandry practices (eg limitation of the vitamin A content of animal feed, restriction of the practice of injecting large doses of vitamin A) in order to reduce rapidly and effectively the vitamin A content of the livers sold for consumption. It would be important to reduce the vitamin A content of the liver to the lowest possible level compatible with the welfare and health of the animals.

5. Until appropriate changes in present day animal husbandry achieve this desired objective, the Committee is of the opinion that liver should not be consumed by women who are, or who might become, pregnant. The Committee regrets to have to advise that such discriminatory action be taken against a food which otherwise possesses undoubted nutritional qualities but sees at present no alternative for achieving adequate health protection of this particular section of the population.

6. The developments regarding the vitamin A level of livers should be followed on a Community basis by instituting co-ordinated analytical investigations. Scientific investigations to clarify the exact nature and size of the risk are also desirable.

7. The risk of hypervitaminosis A may be aggravated because of the free availability of dietary vitamin supplements without medical prescription. The Committee considers it advisable to limit the total daily consumption of these vitamin supplements to the Recommended Daily Allowance. To achieve this it is necessary to ensure that these supplements are labelled appropriately as to their composition and that they carry a warning regarding excessive intake, particularly by women who are, or who might become, pregnant.

8. The Committee urges the Commission to take the necessary steps to inform Member States about the risk of hypervitaminosis A from food and the use of vitamin A and the retinoids for any other purposes.

The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, page 1) to advise the Commission on any problem relating to the protection of the health and safety of persons arising from the consumption of food, and in particular the composition of food, processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminants.

The members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

The Secretariat of the Committee is provided by the Directorate-General for Internal Market and Industrial Affairs of the Commission. Recent Council directives require the Commission to consult the Committee on provisions which may have an effect on public health falling within the scope of these directives.

The present report deals with **foods intended for weight control diets** (opinion expressed on 19 October 1990), **guidelines for the presentation of data on food enzymes** (opinion expressed on 11 April 1991), a **recommendation on cyclamates** (opinion expressed on 21 June 1991) and a **report on the risks of hypervitaminosis A** (opinion expressed on 21 June 1991).

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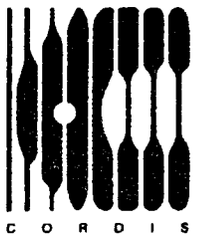


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