Committee considered this information inadequate for making a complete assessment of intake, because no data were provided on the maximum levels of use and the distribution of intake of foods that might contain the additive in different regions of the world.

In two studies, rats given [^{14}C]curdlan at a dose of 20mg/kg of body weight orally excreted about 80% and 40% of the radiolabel as [^{14}C]carbon dioxide within 24 hours, respectively. In these studies, excretion in urine represented about 3% and 1.5% of the dose and excretion in faeces about 8% and 34%, respectively. After 48 hours, 100% and 80% of the radiolabel was recovered from carbon dioxide, urine and faeces combined in the two studies, respectively. When tetracycline was given concomitantly in the drinking-water, excretion as carbon dioxide decreased by one-third, whereas excretion in faeces was increased, indicating that intestinal microflora may be responsible for the metabolism of this compound. Excretion of the radiolabel as carbon dioxide also decreased with increasing dose of curdlan, indicating that metabolism was more limited at higher doses. In humans, the faeces appeared to be the main pathway for excretion, except for a portion that was fully metabolized to carbon dioxide. The extent of metabolism to carbon dioxide in humans also appeared to reflect the action of intestinal bacteria: when the bacterial microflora were suppressed by pretreatment with antibiotics, very limited production of [^{14}C]carbon dioxide was seen.

Curdlan given to rats at concentrations of 10, 50 or 150g/kg of feed had no effect on the bioavailability of calcium, magnesium, iron, zinc, copper or manganese.

The LD_{50} value in mice and rats treated orally was >10g/kg of body weight, and no abnormalities were seen at autopsy.

In short-term and long-term studies in experimental animals, the only effects of orally administered curdlan were soft stools and/or laxation, reduced body-weight gain and increased weights of full and empty caeca due to the presence of high concentrations of undigested curdlan. In an 8-week study in mice and a 4-week study in rats given curdlan at concentrations of up to 300g/kg of feed, the only effects were large faecal pellets, soft stools and/or laxation and increased weights of full and empty caeca.

In a 3-month study in rats, the NOEL was the lowest concentration tested, 50g/kg of feed. Growth was inhibited at the highest level, 200g/kg of feed, even though food intake was increased. Soft stools, enlarged large intestines when full and increased weights of full and empty caeca appeared to be the major effects at 100 and 200g/kg of feed. Dose-related decreases in platelet counts and protein and

globulin concentrations and dose-related increases in serum alkaline phosphatase activity, absolute carcass weight and the relative weights of adrenal glands and submaxillary glands were seen in males at 100 and 200 g/kg of feed. In addition, males in the highest-dose group had decreased serum calcium and cholesterol concentrations, while females had decreased relative pituitary weights and increased relative uterine weights. At necropsy, decreased deposition of adipose tissue was seen in the abdominal cavity in females at all doses and in males at the highest dose.

In a 1-year study in dogs, animals treated with curdlan at a concentration of 150 g/kg of feed or given gelled curdlan at a concentration of 40 g/kg of feed had blood-tinged, mucoid, soft stools. Increases in the weights of full and empty caeca were also observed at 150 g/kg of feed. The petaechial haemorrhages and mucosal ecchymosis occasionally observed in the small intestinal mucosa of dogs at all doses were considered to be unrelated to treatment with curdlan.

In a lifetime carcinogenicity study in mice, addition of gelled curdlan at a concentration of 400 g/kg of feed or of curdlan at concentrations of up to 150 g/kg of feed did not cause any significant abnormalities, although decreased food consumption was seen with curdlan and increased food consumption with gelled curdlan. No changes in tumour incidence were observed.

In a 2-year study in rats, the highest concentration of curdlan (150 g/kg of feed) decreased growth and food consumption and increased the weights of full and empty caeca. Gelled curdlan at 400 g/kg of feed had no effect. A further 2-year study was conducted at the same laboratory, using rats of the same strain. Animals exposed in utero to curdlan at 150 g/kg of feed showed inhibited growth and a slight decrease in food consumption. Increased empty caecal weights were seen in males given curdlan at 50 g/kg of feed and in females at 150 g/kg of feed or given gelled curdlan at 400 g/kg of feed. Clinical chemical analyses during treatment showed increased aspartate aminotransferase and serum alkaline phosphatase activities in animals given curdlan at 150 g/kg of feed or gelled curdlan. Gross and microscopic examination revealed a significantly increased incidence of benign uterine polyps in rats exposed to curdlan at 150 g/kg of feed. The authors reported that benign uterine polyps were seen infrequently in control animals; the incidences in historical controls were not available.

In a three-generation study of reproductive toxicity in rats, with two litters per generation, no effect was seen on fertility, gestation or the viability of the pups. Parents given curdlan at 150 g/kg of feed or gelled curdlan at 400 g/kg of feed showed slight growth inhibition.

Food consumption was slightly decreased in parents given gelled curdlan at 400 g/kg of feed. Furthermore, F_2 dams given curdlan or gelled curdlan had increased full and empty caecal weights. The weights of the pups in most litters of dams given curdlan were significantly decreased during lactation: in F_{1a} and F_{1b} litters at 14 and 21 days of age; in F_{2a} litters at 4, 7, 10, 14, 17 and 21 days of age; in F_{2b} litters at day 4 of age; in F_{3a} litters at 4, 7, 10, 14, 17 and 21 days of age; and in F_{3b} litters at day 21 of age. The NOEL for both maternal toxicity and embryotoxicity was 50 g/kg of feed. Although the authors suggested that the decrease in the weight gain of pups during lactation was due to consumption of the dams' feed, it could have been a treatment-related effect or a combination of consumption of the treated feed and an effect via the milk. In order to investigate these possibilities, a number of single-generation studies (two litters per generation) were performed in which the offspring of treated dams were nursed by untreated dams and the offspring of untreated dams were nursed by treated dams. Another single-generation study was conducted in which rats were given cellulose at 50 or 150 g/kg of feed. Both curdlan and cellulose significantly decreased pup weight gain during lactation at 150 g/kg of feed; this effect was decreased in pups transferred from treated to control dams during lactation. When treatment of the dams with curdlan during lactation was withdrawn, the weights of the pups of treated dams during lactation were comparable to those of the pups of control dams.

The three-generation study of reproductive toxicity included a teratogenicity study in the F_{2c} litters. No embryotoxic or teratogenic effects were observed at any concentration of curdlan up to 150 g/kg of feed or of gelled curdlan up to 400 g/kg of feed. In a teratogenicity study in rabbits treated orally by gavage at up to 5 g/kg of body weight per day, no teratogenic effects were seen.

Curdlan had no effects in in vitro assays for gene mutation in bacteria or mouse lymphoma cells, and the results of chromosomal aberration tests in hamster ovary cells were negative. It did not induce micronucleus formation in mice treated in vivo.

No pathogenic effects were observed in mice that received live or dead cells of the producing microorganism, *Alcaligenes faecalis* var. *myxogenes*, strain NTK-u, IFO 13140, orally or in mice that received intravenous, intraperitoneal or intracerebral injection of live organisms. This curdlan-producing strain was not cytotoxic to HeLa cells.

Curdlan was not immunotoxic in mice or rats. It did not induce skin sensitization in a study in humans, although the study was of limited value.

In a 4-week study in which six volunteers each consumed up to 50 g of curdlan daily, increased flatulence was observed. One subject who consumed 50 g of curdlan per day had some diarrhoea. No evidence of toxicity was seen.

In summary, curdlan did not induce genotoxic, carcinogenic or teratogenic effects or effects on reproduction. At high doses, it decreased growth and/or food consumption and increased the weights of full and/or empty caeca. These effects are commonly observed after the consumption of large amounts of indigestible bulking materials.

The Committee noted the significant increase in the incidence of benign uterine polyps in rats exposed in utero to curdlan at a concentration of 150 g/kg of feed. The effect appeared to be dose-related; however, uterine polyps were not observed in the lifetime study in mice or in the 2-year study in rats of the same strain from the same laboratory that were not exposed in utero. These benign growths are known to occur naturally in older rats at incidences of 1–20%, depending on the study and strain. Taking into consideration the lack of genotoxicity of curdlan and its structure and metabolism, the Committee allocated a temporary ADI "not specified"[1] to curdlan for use as a food additive. The ADI was made temporary, pending the provision of the following information:

- information on the use of curdlan, including the maximum and typical levels expected to occur in the food categories proposed in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants;
- data on the consumption of foodstuffs that might contain curdlan in different regions of the world, to permit assessment of the intake.

This information is required for evaluation in 2001.

A toxicological monograph, including information on intake, and new specifications were prepared.

3.4 Miscellaneous substances

3.4.1 *γ-Cyclodextrin*

γ-Cyclodextrin is a ring-shaped molecule made up of eight glucose units linked by α-1,4-bonds. The circular structure of γ-cyclodextrin provides a hydrophobic cavity that allows incorporation and solubilization of a variety of organic molecules, while the hydrophilic outer surface makes it water-soluble. γ-Cyclodextrin is used as a carrier for flavours, sweeteners and colours. It is also proposed for use as a

[1] See footnote on page 22.

carrier for vitamins and polyunsaturated fatty acids and as a flavour modifier.

γ-Cyclodextrin was previously evaluated by the Committee at its fifty-first meeting (Annex 1, reference *137*). At that meeting, the Committee concluded that there were sufficient data to allocate a temporary ADI "not specified", but that the results of a study of human tolerance known to have been conducted should be reviewed in order to confirm the absence of adverse effects on the gastrointestinal tract at normal levels of intake. The results were required for evaluation in 1999. At its present meeting, the Committee reviewed the results of that study and of a 12-month study of toxicity in rats treated orally, which had also become available.

In the latter study, rats were given γ-cyclodextrin at concentrations of up to 200 g/kg of feed. Minimal changes were seen at the highest dose, probably as a result of the presence of a large amount of an osmotically active substance in the large intestine. These changes were considered to be transient and not of toxicological significance.

The study of adverse effects in humans indicated that γ-cyclodextrin did not cause symptoms of gastrointestinal discomfort when ingested at levels of up to 8 g per serving (equal to 0.11 g/kg of body weight in males and 0.13 g/kg of body weight in females).

The estimated 3-day average daily per capita intake of γ-cyclodextrin when used at a maximum level in 19 foods was 4 g, and the intake by consumers in the 90th percentile was 7.5 g.

On the basis of the above studies, and the information reviewed at its fifty-first meeting, the Committee allocated an ADI "not specified"[1] to γ-cyclodextrin.

An addendum to the toxicological monograph was prepared. The existing specifications were revised, with minor changes.

3.4.2 *Sodium iron EDTA*

Sodium iron(III) EDTA (ethylenediamine tetraacetate or edetic acid) was previously evaluated by the Committee at its forty-first meeting (Annex 1, reference *107*), when it provisionally concluded that use of sodium iron EDTA meeting the tentative specifications prepared at the meeting would not present a safety problem in supervised food fortification programmes in iron-deficient populations. The Committee requested that additional studies be conducted to assess the site of deposition of iron administered in this form and to

[1] See footnote on page 22.

assess the metabolic fate of sodium iron EDTA after long-term administration. The Committee emphasized that its evaluation applied only to the use of sodium iron EDTA as a dietary supplement to be used under supervision, and expressed its concern about the potential for over-fortification of food because of the enhanced bioavailability of iron in this form.

Several studies were submitted in response to the Committee's request, which were reviewed at the present meeting. One study that was specifically designed to address the Committee's concerns involved feeding male rats diets containing iron in two forms, ferrous sulfate and sodium iron EDTA, for 62 days. The dietary concentrations provided iron intakes of 2.8, 5.7 and 12 mg/kg of body weight per day from ferrous sulfate, and 2.8, 5.7 and 11 mg/kg of body weight per day from sodium iron EDTA. There was a dose-related increase in the amount of non-haem iron stored in the liver, spleen and kidney, which was more pronounced in the animals fed diets containing ferrous sulfate. There was no evidence that the total iron-binding capacity of the blood plasma was altered by treatment with sodium iron EDTA. The Committee therefore concluded that there was no evidence that administration of iron in the form of sodium iron EDTA would result in greater uptake of iron than that from an equivalent dietary concentration of ferrous sulfate once the nutritional requirement for iron is satisfied. There was no evidence of adverse effects at the highest daily intake of iron from sodium iron EDTA, i.e. 11 mg/kg of body weight, which is 55 times the proposed daily human intake of 0.2 mg/kg of body weight in food fortification programmes.

Short-term studies in rats and humans have shown no adverse effects of dietary intake of sodium iron EDTA on the concentrations of other minerals such as calcium, copper, manganese and zinc. The results of an intervention study in iron-deficient populations in Guatemala demonstrated the efficacy of a diet supplemented with sodium iron EDTA in reducing the prevalence of iron deficiency. The Committee therefore considered that the data submitted satisfied its concerns about the use of sodium iron EDTA in food fortification programmes.

The Committee was aware of the results of acute toxicity, mutagenicity, teratogenicity and 90-day toxicity studies in rats given sodium iron EDTA. Full reports of these studies were not available to the Committee, but the information was considered unnecessary for evaluating the safety of this compound. The Committee also received an assessment of the potential intake of sodium iron EDTA by consumers in the United States that would result from fortification of foodstuffs. The Committee was of the view that this assessment was

not relevant to any proposed use of sodium iron EDTA as a food fortifier in areas of iron deficiency.

The Committee concluded that sodium iron EDTA could be considered safe when used in supervised food fortification programmes in response to a need for iron supplementation in a population as determined by public health officials. Such programmes would provide a daily iron intake of approximately 0.2mg/kg of body weight.

An addendum to the toxicological monograph was prepared. The existing specifications were revised to include an identification test for sodium, a method for the analysis of nitrilotriacetic acid and a modified method of assay for sodium iron EDTA.

3.4.3 *Sodium sulfate*

Sodium sulfate has not been evaluated previously by the Committee. The sulfate anion was evaluated at the twenty-ninth meeting (Annex 1, reference *70*), when an ADI "not specified" was established, on the basis that sulfate is a natural constituent of food and is a product of sulfur metabolism in animals. Sodium sulfate was not specifically included in that ADI because no information was available to indicate that it was being manufactured or used as a food-grade material. It was evaluated at the present meeting at the request of the Codex Committee on Food Additives and Contaminants because it is included in the draft General Standard for Food Additives.

The Committee was unaware of any data on the dietary intake of sodium sulfate in human populations.

The Committee considered that the results of the published studies in experimental animals did not raise any concern about the toxicity of sodium sulfate. The compound has a laxative action, which is the basis for its clinical use. The minor adverse effects reported after ingestion of purgative preparations containing sodium sulfate may not be due to the sodium sulfate itself.

In the absence of any evidence of toxicity, the Committee allocated a temporary ADI "not specified"[1] to sodium sulfate in accordance with the principles established at its twenty-ninth meeting.

A toxicological monograph and new specifications were prepared. The new specifications were designated as "tentative", pending the submission of information on the functional effect and actual uses of sodium sulfate in foods. This information is required for evaluation in 2001.

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[1] See footnote on page 22.

4. Substances evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

Two groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Fig. 1 (Annex 1, references *116*, *122*, *131* and *137*).

The Committee noted that, in applying the Procedure, the substance is first assigned to a structural class as identified at the forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

- Class I. Substances that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity when given by the oral route.
- Class II. Substances that have structural features that are less innocuous than those of substances in Class I, but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Substances that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference *122*):

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

Endogenous substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These were derived from surveys in Europe and the USA. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents