SAFETY EVALUATIONS OF GROUPS OF RELATED FLAVOURING AGENTS

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INTRODUCTION

Seven groups of flavouring agents were evaluated by the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149* and *154*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference 122). The structural classes are as follows:

- Class I. Flavouring agents that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity by the oral route.
- Class II. Flavouring agents 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. Flavouring agents 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:

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.

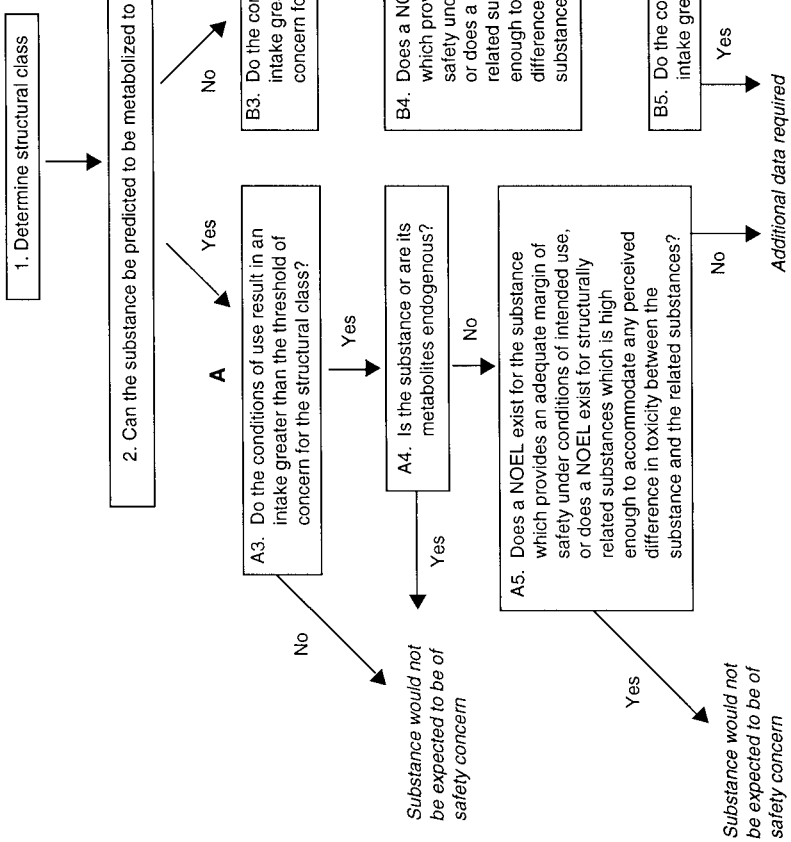
Endogenous substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to,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

Intake

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data 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 the European Union during the previous year. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the National Academy of Sciences National Research Council (under contract to the

Figure 1. Procedure for the safety evaluation of flavouring agents



Food and Drug Administration) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added in a number of broad food categories.

In using the data from these surveys to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported in Europe and 80% of the amount used is reported in the USA and that the total amount used in food is consumed by only 10% of the population.

$$\text{Intake (}\mu\text{g/person per day)} = \frac{\text{annual volume of production (kg)} \times 10^9\ (\mu\text{g/kg})}{\text{population of consumers} \times 0.6 \text{ (or } 0.8) \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

Several of the flavouring agents that were evaluated at the present meeting were not included in the above surveys or were placed on the market after the surveys were conducted. Intakes of these flavouring agents were estimated on the basis of anticipated use by the manufacturer in the USA, and the standard formula was applied.

MALTOL AND RELATED SUBSTANCES

First draft prepared by

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1.0 EVALUATION

1.1 Introduction

The Committee evaluated a group of seven flavouring agents (see Table 1) comprising maltol and related substances. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1, p. 170). The Committee had evaluated two members of the group previously. Maltol (No. 1480) was evaluated at the eleventh meeting (Annex 1, reference 15), when a temporary ADI of 0–1 mg/kg bw was established because no results of long-term studies were available. At its eighteenth meeting (Annex 1, reference 35), the Committee withdrew the temporary ADI because the results of the long-term studies of toxicity that had been requested at its previous meeting had not been made available. At its twenty-second meeting (Annex 1, reference 47), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw.

Table 1. Summary of results of safety evaluations of maltol and related substances used or proposed for use as flavouring agents

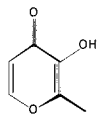
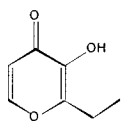
Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II							
Maltol	1480	118-71-8 	Yes Europe: 3585 USA: 2898	No	Yes. The NOEL of 100 mg/kg bw per day (Annex 1, reference 56) is > 1600 times the estimated daily intake of maltol when used as a flavouring agent.	Note 1	At its 25th meeting, JECFA established an ADI of 0–1 mg/kg bw (Annex 1, reference 56).
Ethyl maltol	1481	4940-11-8 	Yes Europe: 1851 USA: 6692	No	Yes. The NOEL of 200 mg/kg bw per day for ethyl maltol in rats (Annex 1, reference 35) is > 1800 times the estimated daily intake of ethyl maltol when used as a flavouring agent.	Note 1	At its 18th meeting, JECFA established an ADI of 0–2 mg/kg bw (Annex 1, reference 35).

Table 1 (contd)

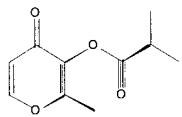
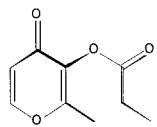
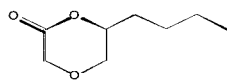
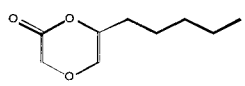
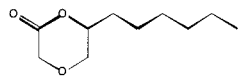
Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Maltyl isobutyrate	1482	65416-14-0 	No Europe: 23 USA: 38	NR	NR	Note 2	No safety concern
2-Methyl-3-(1-oxo-propoxy)-4*H*-pyran-4-one	1483	68555-63-5 	No Europe: ND USA: 26b	NR	NR	Note 2	No safety concern (conditional)
Structural class III 2-Amyl-5 or 6-keto-1,4-dioxane	1485	65504-96-3 	No Europe: ND USA: 0.2	NR	NR	Note 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class III							
2-Butyl-5- or -6-keto-1,4-dioxane	1484	65504-95-2 	No Europe: ND USA: 0.5	NR	NR	Note 3	No safety concern
2-Hexyl-5 or 6-keto-1,4-dioxane	1486	65504-97-4 	No Europe: ND USA: 0.5	NR	NR	Note 3	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation because intake of the substance was determined to be of no safety concern at Step A3 of the procedure.

Step 2: All the agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure.

[a] The thresholds for human intake for structural classes II and III are 540 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intakes of flavouring agents in structural class II are 5459 μg/day in Europe and 9655 μg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 1.2 μg/day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Notes:

1. Conjugation with glucuronic acid or sulfate followed by excretion in urine
2. Hydrolysis to maltol and the corresponding carboxylic acid, followed by conjugation with glucuronic acid or sulfate and excretion in urine
3. Hydrolysis to a hydroxycarboxylic acid, followed by excretion as the glucuronic acid conjugate

At its twenty-fifth meeting (Annex 1, reference 56), the Committee evaluated additional data and assigned an ADI of 0–1 mg/kg bw. Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting (Annex 1, reference 22), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting (Annex 1, reference 35), the Committee re-evaluated ethyl maltol and confirmed the previous ADI of 0–2 mg/kg bw.

One of the seven substances, maltol (No. 1480), has been reported to occur naturally in a wide variety of foods, including wheaten and rye bread, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soya proteins, beans, and clams. Under conditions of baking (e.g. bread, beans) and roasting (cocoa, coffee, peanuts), simple sugars are partly converted to maltol (Nijssen et al., 2003).

1.2 Estimated daily per capita exposure

Annual volumes of production have been reported for six of the seven flavouring agents in the group (Nos 1480, 1481, 1482, 1484, 1485 and 1486). With respect to the remaining substance (No. 1483), anticipated annual volumes of production have been given for its proposed use as a flavouring agent. The total reported and anticipated annual volume of production of the seven flavouring agents in this group is about 38 000 kg in Europe (International Organization of the Flavor Industry, 1995) and 73 000 kg in the USA (National Academy of Sciences, 1970, 1982; Lucas et al., 1999). More than 99% of the total reported and anticipated annual volumes of production in Europe and the USA is accounted for by maltol and ethyl maltol. The per capita intakes of maltol in Europe and the USA are about 3600 and 2900 μg/day, respectively. The per capita intakes of ethyl maltol in Europe and the USA are about 1800 and 6700 μg/day, respectively. The per capita exposure to the remainder of the flavouring agents in the group is 0–23 μg/day in Europe and 0.2–38 μg/day in the USA, most of the values being at the lower end of these ranges. The per capita exposure to each agent is reported in Table 2.

1.3 Absorption, distribution, metabolism and elimination

Chemically, maltol is classified as a γ-pyrone. It is a hydroxyl-substituted 4*H*-pyran-4-one and is expected be metabolized similarly to phenol, primarily undergoing phase II conjugation of the free hydroxy substituent. Maltol (2-methyl-3-hydroxy-4*H*-pyran-4-one) and ethyl maltol (2-ethyl-3-hydroxy-4*H*-pyran-4-one) are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine (Rennhard, 1971). Maltol esters (Nos 1482 and 1483) are predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (propionic acid or isobutyric acid) (Bennett, 1998) and to undergo further metabolism similar to that of maltol and ethyl maltol.

The remaining three substances (Nos 1484, 1485, and 1486) in the group are α-pyrone derivatives and contain a saturated 3*H*-pyranone nucleus. These three substances are lactones and are readily hydrolysed to yield the corresponding ring-opened hydroxy acid derivatives. In nature, lactones are formed by acid-catalysed intramolecular cyclization of four- or five-carbon hydroxycarboxylic acids to yield five- (γ-) or six- (δ-) membered lactone rings, respectively. The stability of the lactone ring in an aqueous environment is pH-dependent. In basic media such as blood, lactones hydrolyse rapidly to the open-chain hydroxycarboxylic acid (Fishbein & Bessman, 1966; Roth & Giarman, 1966; Guidotti & Ballotti, 1970). Studies of

Table 2. Annual volumes of production of maltol and related substances used or proposed for use as flavouring agents in Europe and the USA

Agent (No.)	Reported[a] / anticipated annual volume (kg)	Exposure[b]		Annual volume in naturally occurring foods (kg)[c]	Consumption ratio[d]
		µg/day	µg/kg bw per day		
Maltol (1480)					
Europe	25 123	3 585	60		
USA	21 999	2 898	48	38 694	2
Ethyl maltol (1481)					
Europe	12 969	1 851	31		
USA	50 802	6 692	112	–	NA
Maltyl isobutyrate (1482)					
Europe	163	23	0.4		
USA	286	38	0.6	–	NA
2-Methyl-3-(1-oxopropoxy)-4*H*-pyran-4-one (1483)					
Europe	ND	ND	ND		
USA[e]	150	26	0.4	–	NA
2-Butyl-5- or -6-keto-1,4-dioxane (1484)					
Europe	ND	ND	ND		
USA[f]	3	0.5	0.009	–	NA
2-Amyl-5- or -6-keto-1,4-dioxane (1485)					
Europe	ND	ND	ND		
USA[f]	1	0.2	0.003	–	NA
2-Hexyl-5- or -6-keto-1,4-dioxane (1486)					
Europe	ND	ND	ND		
USA[f]	3	0.5	0.009	–	NA
Total					
Europe	38 255				
USA	73 244				

NA, not available; ND, no intake data reported; –, not reported to occur naturally in foods

[a] From International Organization of the Flavour Industry (1995) and Lucas et al. (1999) or National Academy of Sciences (1970, 1982)

[b] Exposure (µg/person per day) calculated as follows: [(annual volume, kg) x (1×10^9 µg/kg)/(population x survey correction factor x 365 days)], where population (10%, 'eaters only') = 32×10^6 for Europe and 26×10^6 for the USA; where survey correction factor = 0.6 for Europe and 0.8 for the USA, representing the assumption that only 60% and 80% of the annual flavour volume, respectively, was reported in poundage surveys (International Organization of the Flavor Industry, 1995; Lucas et al., 1999; National Academy of Sciences, 1982) or in the anticipated annual volume.
Exposure (µg/kg bw per day) calculated as follows: [(µg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

[c] Quantitative data for the USA reported by Stofberg and Grundschober (1987)

[d] The consumption ratio is calculated as follows: (annual consumption from food, kg)/(most recent reported volume as a flavouring substance, kg)

[e] The volume cited is the anticipated annual volume, which was the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use. National surveys (National Academy of Sciences, 1970, 1982, 1987; Lucas et.al., 1999), if applicable, revealed no reported use as a flavour agent.

[f] Annual volume reported in previous surveys in the USA (National Academy of Sciences, 1970, 1982)

structurally related lactones (Billecke et al., 2000) indicate that the aliphatic lactones would be hydrolysed to yield the corresponding hydroxycarboxylic acid. These acids can undergo further oxidation to yield polar, excretable metabolites or enter the fatty acid pathway and undergo β-oxidative cleavage to yield polar metabolites of lower relative molecular mass, which are also excreted either unchanged or conjugated in the urine (Nelson & Cox, 2000).

1.4 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the intake estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure to this group of flavouring agents, the Committee assigned four of the seven agents (Nos 1480, 1481, 1482 and 1483) to structural class II and the remaining three agents (Nos 1484, 1485 and 1486) to structural class III (Cramer et al., 1978).

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A side of the procedure.

Step A3. The estimated daily per capita exposure to two of the four agents in structural class II (Nos 1482 and 1483) and of all three agents in structural class III is below the threshold of concern for their respective class (i.e. class II, 540 μg/day; class III, 90 μg/day). Four of these five substances (Nos 1482, 1484, 1485 and 1486) are reported to be used as flavouring agents. According to the Procedure, use of these four agents would not raise concern about safety at the estimated daily exposure. The other substance (No. 1483) is proposed for use as a flavouring agent. Although the Procedure indicates no safety concern with use of this flavouring agent at the estimated daily exposure derived from the anticipated annual volume of production, less uncertain exposure estimates are needed. Estimated daily exposure to the remaining two agents in structural class II, maltol (No. 1480) and ethyl maltol (No. 1481), exceed the threshold of concern for structural class II. The per capita exposure to maltol is about 3600 μg/day in Europe and 3000 μg/day in the USA, and the exposure to ethyl maltol is about 1800 μg/day in Europe and 6700 μg/day in the USA. Accordingly, the evaluation of these two agents proceeded to step A4.

Step A4. Maltol (No. 1480) and ethyl maltol (No. 1481) are not endogenous. Therefore, their evaluation proceeded to step A5.

Step A5. At its twenty-fifth meeting, the Committee established an ADI of 0–1 mg/kg bw for maltol (No. 1480) on the basis of a NOEL of 100 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference 56). This NOEL is more than 1800 times the estimated daily exposure to this agent from its use as a flavouring agent in Europe or the USA. At its eighteenth meeting, the Committee established an ADI of 0–2 mg/kg bw for ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference 35). This NOEL is more than 1800 times the estimated daily iexposure to this substance from its use as a flavouring agent in Europe or the USA. The Committee therefore concluded that the exposure to flavours in this group would not raise concerns about safety.

The exposure considerations and other information used to evaluate maltol and six related derivatives according to the Procedure are summarized in Table 1.

1.5 Consideration of combined exposure from use as flavouring agents

In the unlikely event that all four agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the human exposure threshold for class II (540 μg per person per day). All four agents in this group are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Their safety is also indicated by the results of studies on the toxicity of maltol and ethyl maltol. An evaluation of all the data indicates that combined intake would not raise concern about safety.

In the unlikely event that all three agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class III (90 μg per person per day). Their safety is also indicated by the results of studies of toxicity. An evaluation of all the data indicates that combined intake would not raise concern about safety.

1.6 Conclusions

The Committee maintained the previously established ADIs of 0–1 mg/kg bw for maltol and 0–2 mg/kg bw for ethyl maltol. The Committee concluded that use of the flavouring agents in this group of maltol and related substances would not present a safety concern at the estimated daily intakes. For one agent (No. 1483), the evaluation was conditional, because the estimated daily exposure was based on the anticipated annual volume of production. The conclusion about the safety of this substance will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of the maltol derivatives were consistent with the results of the safety evaluation made with the Procedure.

2. RELEVANT BACKGROUND INFORMATION

2.1 Explanation

The relevant background information summarizes the key scientific data applicable to the safety evaluation of seven flavouring agents that include maltol and related substances.

2.2 Additional considerations on exposure

Maltol (No. 1480) is the only substance in this group of flavouring agents that is reported to occur in traditional foods. Quantitative data on natural occurrence and a consumption ratio reported for maltol indicate that exposure is predominantly from consumption of traditional foods (i.e. a consumption ratio > 1) (Stofberg & Kirschman, 1985; Stofberg & Grundschober, 1987). The production volumes and exposure values for each flavouring agent in this group are shown in Table 2.

2.3 Biological data

2.3.1 Biochemical data

(a) Hydrolysis

In general, aromatic esters are hydrolysed in vivo by the catalytic activity of carboxylesterases (Heymann, 1980), the most important of which are the A-esterases. Carboxylesterases are present in the endoplasmic reticulum of most mammalian tissues (Hosokawa et al., 2001), predominantly in hepatocytes (Graffner-Nordberg et al., 1998; Hosokawa et al., 2001).

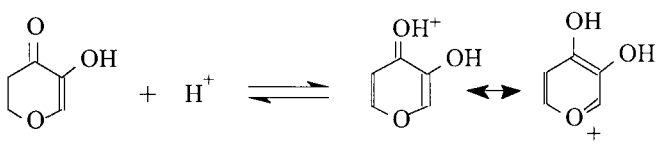
Incubation of 2-methyl-4-pyron-3-yl 2-methylpropanoate (maltol isobutyrate, No. 1482) with simulated gastric and intestinal fluid was reported to result in complete hydrolysis to the corresponding acid (isobutyric acid) and alcohol (maltol) within 10 and 15 h, respectively (Flavor and Extract Manufacturers Association of the United States, 1973). Incubation of maltol propionate (No. 1483) with simulated intestinal fluid containing pancreatin at 37 °C resulted in essentially complete hydrolysis within 5 h (Bennett, 1998).

(b) Absorption, distribution, metabolism and excretion

Maltol (No. 1480) and derivatives (Nos 1481 to 1483) contain a γ-pyrone ring system. γ-Pyrones are relatively basic, and the behaviour as a base is partly due to the aromatic character and relative stability of the conjugate acid (see Figure 1). As that the γ-pyrone ring also contains a 3-hydroxy substituent, it is expected that maltol and its derivatives will be readily conjugated with glucuronic acid or sulfate. In addition, maltol may form a complex with metal ions (e.g. Fe^{++}), like phenols. It also has antioxidant properties in vitro and in vivo (see *Other biochemical properties*, below).

Groups of two beagle dogs of each sex were given a single intravenous injection of 10 mg/kg bw maltol (No. 1480), and urine samples were collected for 72 h. An average of 58.5% of the administered dose was excreted as a mixture of sulfate and glucuronic acid conjugates of maltol. About 98% of the total urinary excretion of conjugates occurred within the first 24 h, males and females excreting

Figure 1. Maltol acid–base reaction



an average of 42% and 73% of the administered dose, respectively. In a parallel study, groups of two beagle dogs of each sex were given a single intravenous injection of 10 mg/kg bw ethyl maltol (No. 1481). Analysis of urine samples collected over 72 h showed that an average of 66.3% of the administered dose had been excreted as a mixture of sulfate and glucuronic acid conjugates of ethyl maltol. About 97% of the total excretion occurred within the first 24 h. During that time, males and females excreted an average of 38% and 91% of the administered dose, respectively (Rennhard, 1971).

One male and one female beagle dog were given oral doses of ethyl maltol at 200 mg/kg per day on 2 consecutive days, and excreta were collected for 24 h after each dose. An average of 64% of each dose was excreted in the urine as either the sulfate (male, 12.9%; female, 11%) or the glucuronic acid (male, 46.6%; female, 57.6%) conjugate within 24 h after each dose. Small amounts of free ethyl maltol (male, 0.12%; female, 0.13%) were also detected in urine, and small amounts (male, 2.23%; female, 0.25%) of free and conjugated maltol were detected in faeces (Rennhard, 1971).

The three dioxane compounds, 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485), and 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) are expected to be metabolized similarly to lactones. Lactones undergo hydrolysis to yield the corresponding ring-opened d-hydroxycarboxylic acid. The three dioxanes in this group hydrolyse to 5-hydroxycarboxylic acid derivatives in which position 3 of the chain is occupied by an oxygen atom. This prohibits participation in the fatty acid metabolism pathway. As these hydrolysis products contain polar oxygenated functional groups, however, they are anticipated to be rapidly absorbed and excreted, either free or in conjugated form. The metabolism of aliphatic and alicyclic lactones that do not undergo γ-oxidation in the fatty acid pathway has been reviewed previously by the Committee (Annex 1, reference 166).

In conclusion, the flavouring agents in this group are anticipated to be rapidly absorbed and metabolized, either by conjugation with glucuronic acid or sulfate, like the maltol derivatives, or by hydrolysis, like the lactone derivatives.

(c) Other biochemical properties

Maltol has antioxidant properties, presumably through its ability to complex metal ions such as Fe^{++} and to promote the formation of reduced glutathione (GSH) (Murakami et al., 2001). Maltol at a concentration of 130 μmol/l inhibited iron-mediated lipid peroxidation and increased scavenging of reactive oxygen species by enhancing the supply of NADPH required for regeneration of GSH. Maltol inhibited the formation of thiobarbituric acid-reactive substances when incubated with rat liver microsomes in the presence of Fe^{++} and ascorbate. Maltol at concentrations of 130–140 μmol/l also effectively inhibited the inactivation of NADP-isocitrate dehydrogenase, the principal NADPH-generating enzyme, by Fe^{++}. Maltol significantly increased the oxidation of Fe^{++}, while dimethylpyrone had no effect. The latter results suggest that the 3-hydroxy substituent in maltol is necessary to promote Fe^{++} oxidation.

Kainic acid has been shown to induce oxidative stress (increased lipid peroxidation and decreased GSH levels) in the brain tissue of rodents, causing neurobehavioural effects (Gupta et al., 2002). In a further study, male ICR mice were given maltol at 0, 50 or 100 mg/kg bw on 5 consecutive days; 30 min after the final administration, the animals were given kainic acid in a single subcutaneous

injection of 50 mg/kg bw. Administration of kainic acid alone resulted in epileptic-like seizures, causing 50% mortality, damage to pyramidal cells of the hippocampus, marked decreases in GSH content and GSH peroxidase activity, and increases in the level of thiobarbituric acid-reactive substances in brain tissue. Administration of maltol at 100 mg/kg bw, but not 50 mg/kg bw, attenuated the neurobehavioural effects, and the loss of neurons in the hippocampus and mortality (12.5%) were significantly reduced. Maltol also restored brain GSH and GSH peroxidase activity to control levels (Kim et al., 2004).

2.3.2 *Toxicological studies*

(a) Acute toxicity

LD_{50} values after oral administration have been reported for three of the seven substances in this group (see Table 3), ranging from 1150 to 2800 mg/kg bw for rats and from 550 to 2100 mg/kg bw for mice. The value for guinea-pigs was 1410 mg/kg bw. These values indicate that the acute toxicity of maltol and related substances after oral intake is low (Dow Chemical Company, 1967; Pellmont, 1968a,b; Gralla et al., 1969; Moreno, 1974a,b).

(b) Short-term studies of toxicity

The results of short-term studies with maltol and related substances are summarized in Table 4.

Maltol (No. 1480)

Mice

Eight female Swiss mice were fed a diet containing 3-hydroxy-2-methyl-4-pyrone (maltol) at a level of 0.5% (w/w) for 21 weeks, calculated to provide an average daily intake of 750 mg/kg bw (Food & Drug Administration, 1993). A

Table 3. Results of studies for acute toxicity of maltol and related substances administered orally

No.	Flavouring agent	Species; sex	LD_{50} (mg/kg bw)	Reference
1480	Maltol	Mouse; F	550	Dow Chemical Co. (1967)
1480	Maltol	Mouse; M	848	Gralla et al. (1969)
1480	Maltol	Rat; F	1410	Dow Chemical Co. (1967)
1480	Maltol	Rat; M	1440	Gralla et al. (1969)
1480	Maltol	Rat; NR	2330	Moreno (1974a)
1480	Maltol	Guinea-pig; M	1410	Dow Chemical Co. (1967)
1481	Ethyl maltol	Mouse; M	780	Gralla et al. (1969)
1481	Ethyl maltol	Rat; M, F	M: 1150 F: 1200	Gralla et al. (1969)
1481	Ethyl maltol	Rat; NR	1220	Moreno (1974b)
1482	Maltyl isobutyrate	Mouse; NR	2100	Pellmont (1968a)
1482	Maltyl isobutyrate	Rat; NR	2800	Pellmont (1968b)

M, male; F, female; NR, not reported

Table 4. Results of short-term studies of toxicity and long-term studies of toxicity and carcinogenicity with maltol and related substances

No.	Substance	Species; sex	No. test groups[a]/ no. per group[b]	Duration (days)	NOEL (mg/kg bw per day)	Reference
Short-term studies						
1480	Maltol	Mouse; F	1/8	147	750[c]	Bhathal et al. (1984)
1480	Maltol	Rat; M, F	1/20	90	< 1000	Gralla et al. (1969)
1480	Maltol	Rat; M, F	1/30	186	500[c]	Dow Chemical Co. (1967)
1480	Maltol	Dog; M, F	3/4	90	< 125	Gralla et al. (1969)
1481	Ethyl maltol	Rat; M, F	3/20	90	< 250	Gralla et al. (1969)
1481	Ethyl maltol	Dog; M, F	3/4	90	< 125	Gralla et al. (1969)
1484	2-Butyl-5- or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	6.59 (M) 7.35 (F)[c]	Posternak (1969a)
1485	2-Amyl-5 or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	6.65 (M) 7.33 (F)[c]	Posternak (1969b)
1486	2-Hexyl-5 or -6-keto-1,4-dioxane	Rat; M, F	1/28	90	< 5.96 (M) < 6.76 (F)	Posternak (1969c)
Long-term studies						
1481	Ethyl maltol	Rat; M, F	3/50	730	200[c]	Gralla et al. (1969)
1481	Ethyl maltol	Dog; M, F	3/8	730	200[c]	Gralla et al. (1969)

M, male; F, female

[a] Total number of test groups does not include control animals.

[b] Total number per test group includes both male and female animals.

[c] Study performed with either a single dose or multiple doses that had no adverse effect. The value is therefore not a true NOEL but is the highest dose tested that had no adverse effects. The actual NOEL might be higher.

concurrent control group was maintained. Food and water were provided ad libitum, and body weights were recorded weekly. At termination, no differences in general health, behaviour, body-weight gain or relative liver weights were reported. Gross and microscopic examination revealed no histological abnormalities in the livers of the treated mice when compared with the controls (Bhathal et al., 1984).

Rats

Groups of 10 Charles River weanling albino rats of each sex were maintained on a diet containing maltol at a level calculated to provide an average daily intake of 1000 mg/kg bw for 90 days. Concurrent control groups of an unspecified number of male and female rats were maintained on basal diet. Body weight and food consumption were recorded weekly. Blood and urine samples were collected from five male and five female rats in each group 45 and 90 days after the beginning of the study. Blood samples were analysed for haemoglobin, erythrocyte volume fraction, red blood cell count, total white blood cell count and differential count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was examined microscopically after centrifugation. At the end of the study, all rats were necropsied; organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries), and gross and microscopic examinations were made of brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland. Decreased body-weight gain was reported in males and females after weeks 3 and 9, respectively, the male rats being more severely affected. A decrease in haemoglobin and slightly amber-coloured serum were observed in one male and one female at study termination. A high incidence of albuminuria was observed in all treated rats. No significant gross pathological changes were detected, and no differences between test and control animals in organ weights were recorded. Microscopic examination revealed kidney lesions identified as dilated acellular glomerular tufts with protein extravasation into Bowman capsules and cast formation within the lumina of dilated corticomedullary tubules. The deaths of two of the treated rats were attributed by the authors to renal failure (Gralla et al., 1969).

Groups of 15 male and 15 female 4- to 5-week-old rats were fed a diet containing 1% maltol for 6 months, calculated to provide an average daily intake of 500 mg/kg bw (Food & Drug Administration, 1993). Concurrent control groups of 15 rats of each sex were maintained on a basal diet. Body weights were recorded twice weekly. The six male and nine female controls and the four male and nine female treated rats that died during the experiment were examined for gross pathological lesions. At study termination, haematological parameters were evaluated in eight treated and control males, and all remaining animals were necropsied. Major organs were examined grossly and weighed, and selected tissues were fixed and stained for microscopic examination. No significant differences in appearance, behaviour, body weights or organ weights were observed between the treated and control animals during the study. The haematological values of treated males were normal

after 6 months. Histopathological examination of the liver, kidney, spleen, adrenals, pancreas and testes of males and females revealed no evidence of lesions that could be associated with treatment. The NOEL was 500 mg/kg bw per day (Dow Chemical Co., 1967).

Dogs

Groups of four male and four female beagle dogs were given capsules containing maltol at a dose of 125, 250 or 500 mg/kg bw per day for 90 days. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, renal function (measured by the bromosulfphthalein excretion test) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were evaluated at the beginning of the study and on days 14, 30, 60 and 90. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid, brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus and ovary) were removed and weighed. Selected tissues (brain, cervical spinal cord, sciatic nerve, hypophysis, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were evaluated microscopically. Three of four animals (sex not specified) at 500 mg/kg bw per day died within 21–41 days, and the fourth was killed when it became moribund. The symptoms before death included weight loss, episcleritis, icteric mucous membranes, emesis, ataxia and prostration. Two dogs (sex not specified) had decreased haemoglobin concentrations, erythrocyte volume fractions and red blood cell counts and increased blood urea nitrogen. Three dogs (sex not specified) had elevated aspartate and alanine aminotransferase activities, and all four (sex not specified) had increased bilirubin levels. Pathological examination of the tissues revealed pulmonary oedema, hepatic and adrenal cortical and medullary necrosis, fatty degeneration of the myocardium and testicular degeneration. Except for slight decreases in haemoglobin, erythrocyte volume fraction, red blood cell count and bilirubin values at 250 mg/kg bw per day, no effects were reported at 125 or 250 mg/kg bw per day. The NOEL was 250 mg/kg per day (Gralla et al., 1969).

Ethyl maltol (No. 1481)

Rats

Groups of 10 Charles River weanling albino rats of each sex were maintained on a diet containing ethyl maltol at levels calculated to provide an average daily intake of 250, 500 or 1000 mg/kg bw for 90 days. Concurrent control groups of unspecified numbers of male and female rats were maintained on a basal diet. Body weight and food consumption were recorded weekly. Blood and urine samples were collected from five male and five female rats from each group 45 and 90 days after the start of the study. Blood samples were analysed for haemoglobin, erythrocyte

volume fraction, red blood cell count, total white blood cell count and differential count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was analysed microscopically after centrifugation. At study termination, all rats were necropsied and organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries); gross and microscopic examinations were made of major tissues (brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland). There were no significant effects on body-weight gain. Two females and three males at the lowest dose had decreased haemoglobin concentration and slightly amber-coloured serum, but these changes were not seen at higher doses. No significant gross pathological changes or changes in organ weights were reported. Microscopic examination revealed a low incidence of kidney lesions, characterized as dilated acellular glomerular tufts with protein extravasation into Bowman capsule and cast formation within the lumina of dilated corticomedullary tubules, in rats at 1000 mg/kg bw per day; however, the incidence was less than that in rats given the same dose of maltol (Gralla et al., 1969).

Dogs

Groups of four male and four female beagle dogs were given capsules containing ethyl maltol at a dose of 125, 250 or 500 mg/kg bw per day for 90 days. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, renal function (bromosulfphthalein excretion test) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were evaluated at the start of the study and on days 14, 30, 60 and 90. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid, brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus, and ovary) were weighed. Selected tissues (brain, cervical spinal cord, sciatic nerve, hypophysis, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were examined microscopically. On day 30, all dogs receiving 500 mg/kg bw per day and half of those receiving 250 mg/kg bw per day showed elevated bilirubin levels, which returned to normal in the dogs at 250 mg/kg bw per day. Microscopic examination of the liver revealed that dogs at 250 mg/kg bw per day had a few or a moderate number of Kupffer cells containing both haemosiderin and small amounts of intracellular bilirubin. In dogs at 125 mg/kg bw per day, a few Kupffer cells contained haemosiderin, but no bilirubin was detected. No other effects were reported (Gralla et al., 1969).

2-Butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) and 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486)

Rats

In three studies, groups of 14 Charles River rats of each sex were fed diets containing 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) or 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) as a 16.7% emulsion in gum arabic for 90 days. The gum mixture was added to the diet at a concentration of 51 mg/kg for the first 4 weeks, 85 mg/kg for weeks 5–10 and 102 mg/kg for weeks 11–13. The doses provided by these concentrations in males and females, respectively, were: 6.59 and 7.35 mg/kg bw per day of 2-butyl-5- or -6-keto-1,4-dioxane, 6.65 and 7.33 mg/kg bw per day of 2-amyl-5- or -6-keto-1,4-dioxane or 5.96 and 6.76 mg/kg bw per day of 2-hexyl-5- or -6-keto-1,4-dioxane. Concurrent control groups (10–14 rats of each sex) were maintained on a basal diet. Body weights and food consumption were recorded weekly. Haematological examinations (haemoglobin concentration, erythrocyte count, erythrocyte volume fraction and total and differential leucocyte counts) and blood urea determinations were performed on 50% of the rats at week 7 and on all rats at week 13. At termination, the livers and kidneys were weighed, and gross and histological examinations were conducted on major organs. Liver, spleen, pancreas, stomach, large and small intestines, epididymis and testicles or ovaries and uterus, kidneys, bladder, heart, lungs, thyroid, adrenal glands, pituitary gland, submaxillary gland, sternal marrow, spinal cord and brain were examined microscopically (Posternak, 1969a,b,c).

In the study with 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), no significant differences were found in body weights between treated and control animals. There was no significant difference in absolute liver weights between the two groups, but a significant increase in relative liver weight was reported in treated males and females. Histopathological examination revealed no evidence of alteration in any organ or tissue (Posternak, 1969a).

In the study with 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485), slight, transient increases in haemoglobin concentration in males and in blood urea in females were reported in week 7 but not at the end of the study. A slight decrease in blood urea in males at week 13 was also reported. Body weights were similar in the two groups. Absolute liver weights were similar in treated and control groups, but an increased relative liver weight was reported in treated males. Histopathological examination revealed no evidence of alteration in any organ or tissue (Posternak, 1969b).

In the study with 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486), increased mean corpuscular haemoglobin was reported in animals of each sex at week 7 and in females also at week 13. In addition, haemoglobin concentrations were increased in treated females at week 7 only. A slight decrease (12.4%) in body weights occurred in treated males in comparison with control males. Absolute kidney and liver weights did not differ significantly between test and control animals; however, mainly because of depressed body weights in males, the relative kidney:body weight ratio was increased in treated males. Histopathological examination of the kidneys revealed pathological lesions in all treated males and females, while control animals had no such lesions. The lesions were less pronounced in females and were characterized by enlargement of the Bowman space and vacuolization of the proximal and distal convoluted tubules. There were no changes in any other organ or tissue examined (Posternak, 1969c).

The effects on relative liver and kidney weights and on clinical chemistry and haematological parameters after consumption of 2-butyl-5- or -6-keto-1,4-dioxane (No. 1484), 2-amyl-5- or -6-keto-1,4-dioxane (No. 1485) or 2-hexyl-5- or -6-keto-1,4-dioxane (No. 1486) were considered to be minimal when the test values were compared with composite rather than individual control groups. The effects were therefore deemed not to be toxicologically significant (Posternak et al., 1969).

(c) Long-term studies of toxicity and carcinogenicity

The results of long-term studies with maltol-related substances are summarized in Table 4.

Ethyl maltol (No. 1481)

Rats

Groups of 25 Charles River weanling albino rats of each sex were maintained on diets containing ethyl maltol at levels calculated to provide an average daily intake of 50, 100 or 200 mg/kg bw per day for 2 years. Body weight and food consumption were recorded weekly. Blood and urine samples were collected at 3, 6, 9, 12, 18 and 24 months. Blood samples were analysed for haemoglobin, erythrocyte volume fraction, red blood cell count and total and differential white blood cell count. Urine samples were analysed for colour, volume, specific gravity, pH, blood, albumin and glucose, and the sediment was examined microscopically after centrifugation. At the end of the study, all rats were necropsied; organ weights were recorded (heart, lungs, liver, kidneys, pancreas, spleen, thymus, mesenteric lymph nodes, adrenals, thyroid, brain, hypophysis, uterus and ovaries), and gross and microscopic examinations were made of major tissues (brain, cervical spinal cord, hypophysis, eye, parotid gland, thyroid and parathyroid, adrenals, thymus, heart, lung, sternum, rib, aorta, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph nodes, reproductive tract, kidneys, urinary bladder, skeletal muscle, femoral nerve, femoral bone marrow, skin and mammary gland). No difference in general health or behaviour was observed between treated and control rats. All rats, including controls, showed a tendency toward albuminuria. Measurements of body weight, haematology, clinical chemistry and histopathology revealed no significant differences between treated and control animals. Neoplasms occurred randomly in test and control animals with no apparent relation between the number, location or type of tumour and treatment with ethyl maltol (Gralla et al., 1969).

Dogs

Groups of eight male and eight female beagle dogs were given capsules containing ethyl maltol at a dose of 50, 100 or 200 mg/kg bw per day for 2 years. Body weights were recorded weekly. Haematological examinations (haemoglobin, erythrocyte volume fraction and red blood cell count), ophthalmological examinations, a renal function test (bromosulfphthalein excretion) and clinical chemistry (blood urea nitrogen, alkaline phosphatase, aspartate and alanine aminotransferases, total bilirubin and glucose) were conducted at 0, 3, 6, 8, 12, 18 and 24 months. At necropsy, major organs (heart, lung, liver, kidneys, pancreas, spleen, thymus, adrenals, thyroid,

brain, pituitary, testes, epididymes, seminal vesicles, prostate, uterus and ovary) were weighed. Selected tissues (brain, cervical spinal cord, hypophysis, sciatic nerve, eye, optic nerve, thyroid and parathyroid, thymus, heart, lung, carinal node, sternum, rib, brachial plexus, aorta, liver, spleen, pancreas, adrenal, stomach, small and large intestine, mesenteric node, all levels of male and female reproductive tracts, kidneys, urinary bladder, femoral bone marrow, skeletal muscle, submaxillary gland, mammary gland and tongue) were examined microscopically. Two dogs per group were killed after 1 year of treatment and the remaining animals at the end of the study. Four dogs (sex not specified) receiving 200 mg/kg per day had slightly elevated serum alanine aminotransferase activity; however, all other measures of liver function were normal, as was liver morphology. At necropsy, pathological and microscopic examination revealed no dose-related effects (Gralla et al., 1969).

(d) Genotoxicity

Two representative flavouring agents in this group have been tested for genotoxicity. The results are summarized in Table 5.

In vitro

Maltol (No. 1480) and ethyl maltol (No. 1481) were weakly mutagenicity (two- to threefold increases in number of revertants) in *Salmonella typhimurium* TA100 at concentrations of 1–3 mg/plate either alone or with an exogenous liver-derived bioactivation system. Activity against TA98 was not detected (Bjeldanes & Chew, 1979). Maltol tested at concentrations of 0.1–10.0 mg/plate increased the number of revertants in strain TA97 at 1 mg/plate by about twofold. No increase was found in the presence of an activation system, or in TA102 alone or with activation (Fujita et al., 1992). In other studies with in *S. typhimurium*, neither maltol (Hayashi et al., 1988; Gava et al., 1989) nor ethyl maltol (Wild et al., 1983) was consistently mutagenic when tested at concentrations up to 10 000 μg/plate alone or in the presence of an activation system.

No evidence of DNA damage was reported when maltol was incubated with *Escherichia coli* strain PQ37 at a concentration of 5 mmol/l (631 μg/ml) for 2 h at 37 °C (Ohshima et al., 1989).

Maltol at concentrations ranging from 0.1 to 1.5 μmol/ml induced sister chromatid exchanges in Chinese hamster ovary cells (Gava et al., 1989) and in human lymphocytes (Jannson et al., 1986; Gava et al., 1989). Gava et al. (1989) suggested that these results were due to an indirect action of maltol and not to its direct reactivity with DNA.

In vivo

When groups of 8-week-old male ddY mice were given a single intraperitoneal injection of 125, 250 or 500 mg/kg bw of maltol and their bone marrow was sampled at 24 h, a dose-dependent increase in the incidence of micronucleated polychromatic erythrocytes was observed at the two highest doses (Hayashi et al., 1988). No evidence of micronucleus formation was reported when ethyl maltol was administered by intraperitoneal injection to groups of 10–14-week-old male and female NMRI mice at a concentration of 420, 700 or 980 mg/kg bw with sampling 30 h later or in

Table 5 .Studies of genotoxicity with maltol and related substances

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
In vitro						
1480	Maltol	Reverse mutation	*S. typhimurium* TA100	4.44 µmol/plate[a] (560 µg/plate)	Negative[b,c]	Kim et al. (1987)
1480	Maltol	Reverse mutation	*S. typhimurium* TA98, TA100	≤ 3 mg/plate (3000 µg/plate)	Positive[d,e]	Bjeldanes & Chew (1979)
1480	Maltol	Reverse mutation	*S. typhimurium* TA92, TA98, TA100, TA104	1.5–11 µmol/plate[a] (189–1387 µg/plate)	Negative	Gava et al. (1989)
1480	Maltol	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	33–10 000 µg/plate	Negative[b,f,g]	Mortelmans et al. (1986)
1480	Maltol	Reverse mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	33–3333 µg/plate	Negative[b,c,g]	Mortelmans et al. (1986)
1480	Maltol	Reverse mutation	*S. typhimurium* TA97, TA102	0.1, 0.5, 1, 5 or 10 mg/plate (100, 500, 1000, 5000 or 10 000 µg/plate)	Weakly positive[b,d,h,i]	Fujita et al. (1992)
1480	Maltol	DNA damage (SOS Chromotest)	*E. coli* PQ37	5 mmol/l[a] (631 µg/ml)	Negative[j]	Ohshima et al. (1989)
1480	Maltol	Sister chromatid exchange	Chinese hamster ovary cells	≤ 1.5 mmol/ml[a] (12.6–189 µg/ml)	Positive[c,k]	Gava et al. (1989)
1480	Maltol	Sister chromatid exchange	Human lymphocytes	≤ 1.0 mmol/l[a] (126.11 µg/ml)	Positive	Jansson et al. (1986)
1481	Ethyl maltol	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	≤ 3.6 mg/plate (3600 µg/plate)	Negative[b,d,l]	Wild et al. (1983)
1481	Ethyl maltol	Reverse mutation	*S. typhimurium* TA98, TA100	≤ 2 mg/plate (2000 µg/plate)	Positive[d,e]	Bjeldanes & Chew (1979)
In vivo						
1480	Maltol	Micronucleus formation	ddY mouse bone marrow	125, 250 or 500 mg/kg	Positive[m]	Hayashi et al. (1988)

Table 5 (contd)

No.	Agent	End-point	Test object	Dose or concentration	Results	Reference
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	6000 ppm (6000 µg/ml)	Equivocal[n]	Zimmering et al. (1989)
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 000 ppm (10 000 µg/ml)	Negative[m]	Mason et al. (1992)
1480	Maltol	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 000 ppm (10 000 µg/ml)	Negative[n]	Mason et al. (1992)
1481	Ethyl maltol	Micronucleus formation	NMRI mouse bone marrow	420, 700 or 980 mg/kg	Negative[m]	Wild et al. (1983)
1481	Ethyl maltol	Micronucleus formation	NMRI mouse bone marrow	980 mg/kg	Negative[m,o]	Wild et al. (1983)
1481	Ethyl maltol	Sex-linked recessive lethal mutation (Basc test)	*Drosophila melanogaster*	14 or 50 mmol/l[p] (1962 or 7007 mg)	Negative[n]	Wild et al. (1983)

[a] Calculated from relative molecular mass of maltol = 126.11
[b] Assay performed with pre-incubation method
[c] Without metabolic activation
[d] With and without metabolic activation
[e] Dose-related mutagenic activity reported only in TA100
[f] With metabolic activation
[g] Dose-dependent increase in number of revertants observed, but the number of revertants was less than twofold higher than that in negative controls.
[h] Weak mutagenicity reported in TA97 only without metabolic activation
[i] Maltol was nitrosated (incubated for 60 min at 37 °C with 25 mmol/l sodium nitrite) before the SOS Chromotest was performed.
[j] Cytotoxicity observed at highest dose
[k] Weak mutagenicity in TA100 at concentrations > 1000 µg/plate, but significant increases not reproducible
[l] Cytoxocoty observed at highest dose
[m] Administered by intraperitoneal injection
[n] Administered orally
[o] Modified test with expression times of 24, 48 and 72 h after treatment
[p] Calculated from relative molecular mass of ethyl maltol = 140.14

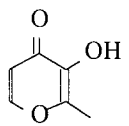
a modified test with sampling 24, 48 or 72 h after treatment with 980 mg/kg bw (Wild et al., 1983).

Equivocal results were obtained for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* larvae fed a concentration of 6000 mg/kg (Zimmering et al., 1989). On the basis of new data and a re-examination of the criteria used to determine mutagenicity in the published data, Mason et al. (1992) reported that maltol did not induce sex-linked recessive lethal mutations in *Drosophila* at concentrations up to 10 000 mg/kg. Ethyl maltol did not induce sex-linked recessive lethal mutations when fed to *D. melanogaster* larvae at concentrations of 14–50 mmol/l (Wild et al., 1983).

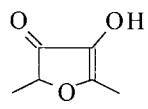
Conclusion

Equivocal or weakly positive results were obtained with maltol and ethyl maltol in some tests for genotoxicity in vitro, and positive or equivocal results were found in vivo with maltol but not with ethyl maltol. The mechanism of the effects is not known, but there is a structural similarity between maltol, ascorbic acid and 4-hydroxy-3-(2*H*)furanones (see Figure 2), which also contain an oxidizable enol functionality. In the presence of metals (e.g. Fe^{3+}) and dissolved oxygen, the enolic OH of ascorbate is oxidized by single-electron transfer to yield the corresponding carbon-centred radical and a reduced metal ion (e.g. Fe^{2+}). The carbon-centred radical can couple to molecular oxygen to produce a peroxyl radical, which might damage DNA. Alternatively, the reduced metal ion could autoxidize to form superoxide radical anion. Superoxide radical would then dismutate into hydrogen peroxide (H_2O_2). It is well recognized that reduced metals react with H_2O_2 to form the hydroxyl radical, which is a powerful oxidizing agent that might cause DNA strand breaks (see Figure 3).

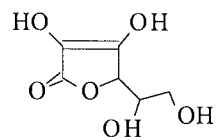
Figure 2. α-Hydroxyenol derivatives



Maltol

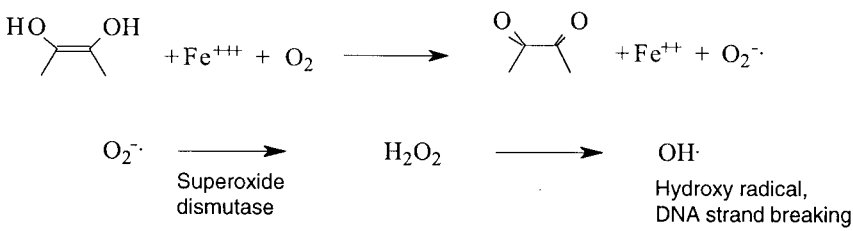


2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone



Ascorbic acid

Figure 3. Mechanism of oxidation of α-hydroxyenol derivatives in vitro



Hydrogen peroxide also oxidizes glutathione, leading to decreased GSH:GSSG and increased cellular oxidative stress.

Ascorbic acid is genotoxic in test systems similar to those in which maltol gave positive results. It induces reverse mutations in *S. typhimurium* strains TA104, TA102, TA100 and TA98 at concentrations of 352–1761 μg/plate (Ichinotsubo et al., 1981; D'Agostini et al., 2000). Increased levels of micronuclei were observed when ascorbic acid (400, 500 or 600 μg/ml) was incubated with Chinese hamster cells (Miller et al., 1995), and increased sister chromatid exchange was observed in Chinese hamster ovary cells in the presence of 500 μg/ml of ascorbic acid without metabolic activation (Tennant et al., 1987). In a standard assay for micronucleus formation in mouse bone marrow, 1500 mg/kg bw ascorbic acid induced a significant increase in B6C3F_1 mice (Shelby et al., 1993). The structural similarity of maltol to ascorbate suggests that a similar mechanism might be responsible for its mutagenicity, although this has not been tested experimentally.

In animals, absorbed maltol is rapidly conjugated with glucuronic acid and sulfate in the liver (see section 1.3). Only minute amounts of free maltol and ethyl maltol are detected in the urine of rats or dogs given high doses; most of an administered dose of maltol or ethyl maltol is rapidly excreted as glucuronic acid and sulfate conjugates in urine. Therefore, despite the structural similarity of maltol and ascorbate, it seems unlikely that the mutagenic activity of maltol would be expressed under the conditions of oral human intake.

(e) Other relevant studies

Maltol (No. 1480)

A three-generation study of reproductive toxicity was conducted, in which groups of 20 male and 20 female rats were given diets containing maltol at concentrations resulting in 100, 200 or 400 mg/kg bw per day. On day 134, animals of the F_1 generation showed signs of sialodacryodenitis due to a contagious virus. No deaths occurred, and the signs diminished within 10 days. Maltol had no effect on copulation rate, mating viability index, lactation, offspring sex ratio or 21-day pup survival index. Discrepancies in F_1 growth rates appeared to be related to the sialodacryodenitis outbreak in the colony. No maltol-related abnormalities or lesions were reported in pups. F_1 pups were weaned and then maintained on the same maltol-containing diets, and F_1 rats were mated on days 189 and 245 to provide the F_{2a} and F_{2b} generations. On day 418, all animals showed signs of sialodacryodenitis. No deaths occurred, and the signs diminished within 10 days. Pup survival was comparable to that of controls. The results of ophthalmic examinations performed during months 12, 18 and 24 were unremarkable. At autopsy, haematological comparisons of test and control animals revealed significant increases in K^+, Cl^-, urea and bilirubin concentrations in males and females at the highest dose. Increased urea and K^+ were found in males at the intermediate dose. No compound-related lesions were found in organs or tissues of the F_{2a} and F_{2b} generations. There was no indication that maltol affected tumour incidence (Annex 1, reference 57).

In a study conducted to develop a long-term animal model of the toxicity of aluminium administered intravenously, groups of 15–16 young adult New Zealand white male rabbits were given 0.075 mmol aluminium maltol or 0.675 mmol maltol (85 mg or approximately 21.3 mg/kg bw) three times per week for 8–30 weeks. A

concurrent control group of 15 animals was maintained. The rabbits were fed standard rabbit chow and had access to food and water ad libitum. All animals were weighed weekly and observed daily for changes in general appearance, food and water consumption and urine and faecal production. The rabbits were also monitored for signs of weakness or loss of neurological function. A statistically insignificant weight gain was reported in the maltol-treated animals. No treatment-related changes in blood chemistry, histology or neurological function were reported in the maltol-treated animals during the study (Berthole et al., 1989).

Ethyl maltol (No. 1481)

Groups of 25 Charles River weanling albino rats of each sex were maintained on a diet containing ethyl maltol at levels calculated to result in an average daily intake of 50, 100 or 200 mg/kg bw for 2 years. Ten pairs of rats at each dose were mated between weeks 15–21 and weeks 30–36. The offspring were killed at weaning. In the parental group, five of each sex were killed after 1 year and the remaining five of each sex at the end of the study. Gross and microscopic examination of parents and offspring indicated no significant effects on fertility, gestation, parturition, lactation or fetal development. Treated and control animals observed for 2 years showed no differences in general health or behaviour, body weight, haematology, clinical chemistry, histopathology results or the incidence of neoplasms (Gralla et al., 1969).

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