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Review Section

Erythritol: An Interpretive Summary of Biochemical, Metabolic, Toxicological and Clinical Data

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Summary—A critical and comprehensive review of the safety information on erythritol was undertaken. Numerous toxicity and metabolic studies have been conducted on erythritol in rats, mice and dogs. The toxicity studies consist of long-term feeding studies conducted to determine carcinogenic potential, intravenous and oral teratogenicity studies to determine the potential for effects on the foetus, oral studies in which erythritol was administered over one or two generations to determine the potential for reproductive effects, and studies in bacterial and mammalian systems to determine mutagenic potential. The majority of the safety studies conducted were feeding studies in which erythritol was mixed into the diet at concentrations as high as 20%. The metabolic studies in animals have shown that erythritol is almost completely absorbed, not metabolized systemically and is excreted unchanged in the urine. The safety studies have demonstrated that erythritol is well tolerated and elicits no toxicological effects. The clinical program for erythritol involved a series of single-dose and repeat-dose, short-duration studies which have been used to investigate the human correlates to the physiological responses seen in the preclinical studies. The clinical studies showed erythritol to be well tolerated and not to cause any toxicologically relevant effects, even following high-dose exposure. Erythritol administered orally to humans was rapidly absorbed from the gastrointestinal tract and quantitatively excreted in the urine without undergoing metabolic change. At high oral doses, urinary excretion accounted for approximately 90% of the administered dose with minimal amounts appearing in the faeces. A comparison of the human and animal data indicated a high degree of similarity in the metabolism of erythritol and this finding supports the use of the animal species used to evaluate the safety of erythritol for human consumption. It can be concluded, based on the available studies that erythritol did not produce evidence of toxicity.

Keywords: erythritol; safety; review; toxicology; metabolism; clinical studies.

Abbreviations: ALP = alkaline phosphatase; APTT = activated partial thromboplastin time; BUN = blood urea nitrogen; GCP = Good Clinical Practices; GLP = Good Laboratory Practices; GGT = γ-glutamyl transferase; HBA = 3-hydroxybutyric acid; LAP-C = leucine aminopeptidase; NAG = *N*-acetyl glucosaminidase; NEFA = non-esterified fatty acids.

Introduction

This review presents the safety database on erythritol, a 4-carbon sugar alcohol. Owing to its unique chemical properties, erythritol is intended for use primarily as a low-calorie sweetener. It has a sweetness 60–80% that of sucrose (Goossens and Röper, 1994; Sugita and Yamazaki, 1988) and is produced from corn or wheat starch by enzymatic hydrolysis yielding glucose which is fermented by safe and suitable food-grade osmophilic yeast, either *Moniliella pollinis* or *Trichosporonoides megachlien-*

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sis. Once erythritol is separated from the fermentation broth, it is purified to result in a crystalline product that is more than 99% pure. Its intended uses principally include confectionery, chewing gum, and beverage and bakery products.

Erythritol occurs widely in nature and has been found to occur naturally in several foods including wine, sake, beer, water melon, pear, grape and soy sauce at levels up to 0.13% (w/v) (Dubernet *et al.*, 1974; Onishi and Saito, 1959, 1960; Shindou *et al.*, 1988, 1989; Sponholz and Dittrich, 1985; Sponholz *et al.*, 1986).

Evidence indicates that erythritol also exists endogenously in the tissues and body fluids of humans and animals (Goossens and Röper, 1994; Horning *et al.*, 1974; Noda *et al.*, 1994; Oku and Noda, 1990a,b; Spencer, 1967). It has been identified in human plasma at levels of approximately 1.2 mg/litre (Niwa *et al.*, 1993) as well as in foetal blood of animals (Britton, 1967; Roberts *et al.*, 1976). Erythritol occurs normally in human urine (Goossens and Röper, 1994). Urinary concentrations have been reported to range from 10 to 100 mg/litre (Pitkänen and Pitkänen, 1964) or 42 to 65 mg creatinine/g (Pfaffenberger *et al.*, 1976).

This critical and comprehensive review of the safety database on erythritol provides a well documented basis from which a safety evaluation of this product can be conducted. Numerous acute, subchronic, chronic and special toxicity studies have been conducted in rats, mice and dogs. The special studies consist of long-term feeding studies conducted to determine carcinogenic potential, intravenous and oral teratogenicity studies to determine the potential for effects on the foetus, oral studies administered over one or two generations to determine the potential for reproductive effects, and studies in bacterial and mammalian systems to determine mutagenic potential. The majority of the safety studies conducted were feeding studies in which erythritol was mixed into the diet at concentrations as high as 20%. All of the safety studies on erythritol have demonstrated that it is well tolerated and elicits no adverse toxicological effects. As erythritol is not systemically metabolized, some intravenous studies also have been conducted. Even when administered intravenously, erythritol did not produce any adverse toxicological effects. The safety database on erythritol provides no evidence that erythritol has any carcinogenic, mutagenic or teratogenic potential. In addition, no effects on reproductive performance or fertility have been reported in the studies.

In animals, at high doses (e.g. 20 g/kg body weight/day), erythritol treatment has been associated with some physiological changes consisting of transient diarrhoea, increased caecal and kidney weights, increased water consumption and urine output, and minor changes in certain urinary parameters. These effects are considered to be physiological responses (WHO, 1987), and are commonly observed in test animals treated with high oral doses of poorly metabolized, or poorly absorbed, low molecular weight compounds, including various carbohydrate compounds. Most importantly, no histopathological evidence of toxicity has been reported in any study conducted with erythritol.

Absorption, distribution, metabolism and excretion studies

Absorption

Erythritol is absorbed from the proximal intestine by passive diffusion in a manner similar to that of many low molecular weight organic molecules which do not have associated active transport systems. The rate of absorption of these types of molecules is related to their molecular size. Consequently, erythritol, a 4-carbon molecule, passes through the intestinal membranes at a faster rate than larger molecules such as mannitol or glucose (Fordtran *et al.*, 1965; Ross *et al.*, 1972; Yuasa *et al.*, 1989). The extent of the *in vivo* absorption of erythritol can be quantified easily since the absorbed fraction does not undergo systemic metabolism and is excreted unchanged in the urine. As a result, quantification of urinary excretion of erythritol is generally representative (excretion in the bile is comparatively small at less than 1% of the administered dose) of fractional absorption provided that the urinary collection period is of sufficient length (generally greater than 24 hours). A summary of the extent of urinary excretion of erythritol in animals and humans is presented in Tables 1 and 2.

Studies in rats and dogs have examined the effects of dose, presence of colonic bacteria, and pre-adaptation on the degree of absorption and excretion of erythritol (Dean *et al.*, 1996; Lina *et al.*, 1996; Nakayama, 1990a,b; Noda and Oku, 1990; Noda *et al.*, 1996; Oku and Noda, 1990a,b; Til *et al.*, 1996; van Ommen and de Bie, 1990; van Ommen *et al.*, 1996). The results of these studies demonstrate a high degree of absorption for erythritol. Unabsorbed erythritol is transported to the large intestine where it is subject to microbial fermentation to volatile short-chain fatty acids, principally acetic, propionic and butyric acids.

In the first of a series of studies conducted with Wistar rats, Oku and Noda (1990a) reported that following administration of erythritol in the diet to groups of five Wistar rats, approximately 94% of the dose was recovered in the urine and 1% in the faeces of animals fed 1 or 5% erythritol in the diet for 8 days. Urinary excretion of erythritol administered at a dietary concentration of 10% was reported to be lower at 83%, but faecal excretion remained unchanged at 1%.

Table 1. Summary of animal studies conducted with erythritol that relate to excretion

Species and conditions	Sex	Dose (mg/kg body weight/day)	Urinary excretion as percent of dose	Faecal excretion as percent of dose	Reference
Mouse		5% diet (6,800)[a]	85.1	7.2	Til *et al.*, 1992, 1996
days 69/70 (m) and 76/77 (f) of subchronic study	m	10% diet (13,800)[a]	95.7	5.3	
		20% diet (35,400)[a]	102.7	4.3	
	f	5% diet (6,600)[a]	57.9	5.3	
		10% diet (15,600)[a]	79.1	3.5	
		20% diet (36,500)[a]	84.7	2.8	
Rat		5% diet (2,260)[b]	60.5	6.6	Til *et al.*, 1991, 1996
days 38/39 of subchronic study	m	10% diet (5,300)[b]	51.6	3.7	
		20% diet (10,150)[b]	54.6	2.3	
		5% diet (1,980)[b]	72.8	3.6	
day 66/67 of subchronic study	m	10% diet (4,500)[b]	57.2	3.0	
		20% diet (11,500)[b]	55.2	4.9	
Rat		2% diet (620)[c]	58.6	2.4	Lina *et al.*, 1994
week 26 of chronic study	m	5% diet (1,700)[c]	61.6	1.5	
		10% diet (3,600)[c]	54.9	1.4	
week 50 of chronic study		2 % diet (460)[c]	62.2	2.9	
	m	5% diet (1,200)[c]	63.4	3.7	
		10% diet (2,800)[c]	64.8	1.4	
week 78 of chronic study		2% diet (370)[c]	118.5	4.1	
	m	5% diet (1,000)[c]	73.5	1.7	
		10% diet (2,700)[c]	59.9	1.4	
week 102 of chronic study		2% diet (370)[c]	64.7	1.1	
	m	5% diet (900)[c]	57.7	0.3	
		10% diet (1,900)[c]	52.4	2.8	
Rat		1% diet	94	2	Oku and Noda, 1990a,b
			94		
			83		
4 days' adaptation and 4 days' feeding	m	5% diet		1	
		10% diet		< 1	
Rat					
single gavage dose ^{14}C erythritol, germfree, not adapted	m and f	101 mg/kg body weight	72.7	13.2	Van Ommen and de Bie, 1990
	m and f	102 mg/kg body weight	81.4	7	
conventional, not added	m and f	106 mg/kg body weight	72.6	6.3	
conventional, adapted for 3 weeks	m and f	99 mg/kg body weight	67	6.7	
Rat		10 mg/kg body weight	91.1	0.04	Noda and Oku, 1992
fasted, single gavage of ^{14}C erythritol	m	100 mg/kg body weight	87.9	0.4	
		1000 mg/kg body weight	65.3	0.6	
conventional, not added	m and f	106 mg/kg body weight	72.6	6.3	
conventional, adapted for 3 weeks	m and f	99 mg/kg body weight	67	6.7	
		1000 mg/kg body weight	65.3	0.6	
adapted to 10% erythritol diet for 2 weeks	m	100 mg/kg body weight	80		
Rat					
single oral gavage dose of ^{14}C erythritol	m	1000 mg/kg body weight	92.7	1.2	Noda *et al.*, 1996
Dog					
single oral gavage dose of ^{14}C erythritol	m	1000 mg/kg body weight	94	0.2	Noda *et al.*, 1996 Nakayama, 1990a

[a]Dose values based on 2 days of food consumption while in metabolic cages and on group mean body weight values at the end of the urine collection period. Overall intakes were estimated by Til *et al.* (1996) to be 44,000 and 45,000 mg/kg body weight/day in high-dose male and female mice, respectively. No estimate was provided for the low- or mid-dose groups.

[b]Dose values based on 2 days of food consumption while in metabolic cages and on group mean body weight values on day 22 (for day 38/39 evaluations) and on day 70 (for day 66/67 evaluations). Overall erythritol intakes for the high-dose male rats were reported to average 12,000 mg/kg body weight/day (Til *et al.*, 1996). Overall average intakes for the low- and mid-dose groups were not reported.

[c]Dose values based on 2 days of food consumption while in metabolic cages and on group mean body weight values for the body weight sampling period prior to entering the metabolic cages. Only males were placed

Table 2. Summary of clinical trials conducted with erythritol that relate to excretion

Subjects	Oral dose	Collection time (hours)	Average percent recovered dose[1] (range)	Reference
Five healthy male subjects	10 g (0.16 g/kg body weight) single dose in solution	0–3 3–8 8–24	41 (34–48) 69.9 (65–75) 91.8 (85–99)	Noda *et al.*, 1988
	20 g (0.34 g/kg body weight) single dose in solution	0–3 3–8 8–24 24–48	39 (29–51) 66.1 (54–85) 86.7 (77–89) 90.5 (81–103)	
Five healthy male subjects	17.3 g (0.3 g/kg body weight) single dose in solution following 12 hr fast	0–3 3–8 8–24 24–48	35 62 85 90.3 ± 4.5	Noda *et al.*, 1994
Four male, one female NIDDM[2] patients	20 g single dose in solution following overnight fast	0–24	85.3 (80–90)	Ishikawa *et al.*, 1996
		24–48 48–72	90.5 (86–97) 91.2 (87–98)	Ishikawa *et al.*, 1992
Four male, two female healthy subjects	25 g single dose in solution following overnight fast	0–6 6–24	52.2 ± 2.5 84.1 3.3	Hiele *et al.*, 1993
Three male, three female healthy subjects	1 g/kg body weight single dose in solution following overnight fast	0–2 0–3 0–24	20 (15–23) 30 (24–36) 78 (60–90)	Bornet *et al.*, 1996a
Three male, three female healthy subjects	0.4 g/kg body weight single dose in chocolate, 2 hours after breakfast	0–2 2–4 4–6 6–8 8–22	(3–41) (9–53) (20–63) (25–69) (45–86) 60.9 ± 13.7 after 22 hours	Bornet *et al.*, 1996b
Four male, two female healthy subjects	0.8 g/kg body weight single dose in chocolate	0–2 2–4 4–6 6–8 8–22	9–15 24–31 26–48 34–61 51–76 61.7 ± 8.2 after 22 hours	Bornet *et al.*, 1996b
12 healthy male subjects	1 g/kg body weight administered in food products, five portions per day with three meals and two snacks	0–24	78 (61–88)	Tetzloff, 1996 Tetzloff *et al.*, 1996

[1]From urine.
[2]NIDDM = non-insulin dependent diabetes mellitus.

In the second study, Noda and Oku (1990, 1992) reported that following a single administration of ^{14}C-erythritol via gavage to male Wistar rats at a dose of 0.1 g/kg body weight, approximately 88% of the dose was recovered in the urine and 6% expired as CO_2 within 24 hours of dosing. The proportion excreted as CO_2 was shown to increase to about 10% when the rats treated at the same dose level were allowed to become adapted to erythritol prior to the conduct of the metabolic study (Noda and Oku, 1992). Also, in non-adapted rats, as the single gavage dose of erythritol was increased from 0.01 g/kg body weight to 0.1 and 1.0 g/kg body weight, the proportion of the dose excreted in the urine decreased from $91.12 \pm 3.36\%$ to $87.94 \pm 5.09\%$, and to $65.29 \pm 5.38\%$, respectively, while the proportion excreted as CO_2 increased from $4.28 \pm 0.62\%$ at a dose of 0.01 g/kg body weight to $5.97 \pm 1.83\%$ and $16.16 \pm 3.81\%$ at doses of 0.1 and 1.0 g/kg body weight, respectively.

In the third study, the high degree of absorption of erythritol was further demonstrated by the finding of 75.3, 91.0, 92.6 and 92.7% of a single oral administration of 1 g ^{14}C-erythritol/kg body weight in the urine of Wistar rats after 8, 24, 72 and 120 hours, respectively (Noda *et al*., 1996). In this study, 4.8% of the dose was found to be excreted in expired air after 120 hours of observation.

Studies in Wistar rats performed by van Ommen and de Bie (1990) and published by van Ommen *et al*. (1996) have further demonstrated that erythritol is well absorbed in germfree rats, conventional rats pre-adapted to dietary erythritol, and in conventional rats not pre-adapted to the consumption of dietary erythritol. Following administration of a 10% solution of U-^{14}C-erythritol, providing an oral dose of approximately 100 mg/kg body weight to groups of three Wistar rats of each sex, van Ommen and de Bie (1990) and van Ommen *et al*. (1996) reported that $72.7 \pm 6.5\%$, $67.0 \pm 6.0\%$ and $72.6 \pm 4.2\%$ of the administered radioactivity was recovered in the urine 24 hours after dosing in the germfree, conventional-adapted, and conventional non-adapted rats, respectively. Faecal excretion in the germfree rats was increased compared with either group of conventional rats (13.2% *v*. 6.3 to 6.7%) while excretion of radioactivity in expired air was comparatively decreased (0.8% *v*. 6.7 to 10.9%).

An absorption/excretion study in beagle dogs demonstrated that, as with rats, erythritol is well absorbed in dogs and excreted largely in the urine (Nakayama, 1990a; Noda *et al*., 1996). Following a single oral administration of 1 g ^{14}C-erythritol/kg body weight, beagle dogs were reported to excrete 44.4, 94.0 and 97.2% of the dose in the urine over 8, 24 and 120 hours, respectively. Only small amounts (0.3% and 1.1%) of the dose were excreted in the faeces and in expired air, respectively (Nakayama, 1990b; Noda *et al*., 1996). In a 1-year toxicity study in which dogs were administered erythritol at levels of 2, 5 or 10% in the diet, 24-hour urinary excretion measured at several time points was reported to account for 83, 56 and 54% of the administered dose, respectively, suggesting a potential dose-dependent decrease in the percentage of the dose absorbed at high doses (Dean *et al*., 1996). Biliary excretion of erythritol in dogs had been estimated earlier to be near 1% using a bile-cannulation method (Lewis *et al*., 1982).

Studies involving humans have demonstrated that most (60 to > 90%) of ingested erythritol is rapidly absorbed via the small intestine and is quantitatively excreted in the urine with only small amounts excreted in the faeces (Bornet *et al*., 1992, 1996a,b; Hiele *et al*., 1993; Höber and Höber 1937; Lauwers *et al*., 1985; Noda *et al*., 1988, 1994; Oku and Noda, 1990a; Tetzloff *et al*., 1996; Winne *et al*., 1985, 1987). The apparent variability in the amount of ingested erythritol absorbed is highly dependent on the urinary collection period. In order to provide a more accurate picture of absorption, a minimum 24-hour collection period is required. Collections taken between 24 and 48 hours show that the 90% value is much more representative of the actual amount of ingested erythritol absorbed.

In a study of two groups of five healthy males who consumed a 20% solution of erythritol to provide single doses of either 10 or 20 g, cumulative urinary excretion was reported to be approximately 40% of the dose within 3 hours, with this total rising to about 70% of the dose within 8 hours in both dose groups (Noda *et al*., 1988). More than 90% of the dose was recovered in the urine within 24 hours for the 10 g dose group and within 48 hours for the 20 g dose group. In another similar study (Noda *et al*., 1994) in which five healthy male volunteers consumed 0.3 g/kg body weight of a 20% erythritol solution, erythritol was found to be rapidly excreted unchanged in the urine with $85.8 \pm 4.6\%$ and $90.3 \pm 4.5\%$ of the dose collected in the urine within 24 and 48 hours, respectively (Noda *et al*., 1994).

Several other human studies investigating the pharmacokinetics of erythritol also have reported high rates of absorption as measured by the degree of urinary excretion (Bornet *et al*., 1996a,b). Bornet *et al*. (1996a) reported that following a single oral dose of 1 g erythritol/kg body weight in aqueous solution to three male and three female fasted subjects, 30% of the dose was recovered in the urine as unchanged compound within 3 hours. Renal excretion increased to an average of 78% following 24 hours of observation. In a subsequent study evaluating the gastrointestinal response and plasma and urine kinetics of erythritol (Bornet *et al*., 1996b), approximately 60% of a single oral 0.4 or 0.8 g/kg body weight dose of erythritol was excreted within 24 hours.

In diabetics, erythritol also has been shown to be rapidly absorbed and excreted unchanged in the urine. In five otherwise healthy patients with non-insulin-dependent diabetes, average age of 52 years and a mean disease-presence of 5 years, $82.0 \pm 3.7\%$ and $88.5 \pm 3.3\%$ of a 20 g dose of erythritol consumed in a liquid solution were detected as parent compound in the urine 24 and 72 hours post-dosing, respectively (Ishikawa *et al.*, 1992, 1996).

Distribution

The distribution and plasma kinetics of erythritol have been evaluated in a number of animal studies (Nakayama, 1990a,b,c,d; Noda *et al.*, 1996). These studies have shown that erythritol, like many other rapidly absorbed low molecular weight polar compounds, attains a maximal plasma concentration shortly after ingestion, of the order of 0.5–1 hour. As would be expected for this type of substance, no great species differences have been noted for the distribution characteristics of erythritol.

Following absorption, ingested erythritol is rapidly distributed throughout the body and has been reported to occur in hepatocytes, pancreatic cells, and vascular smooth muscle cells (Alpini *et al.*, 1986; de Pont *et al.*, 1978; Dewhurst *et al.*, 1978; Johansson, 1969; Jonsson, 1971; Lake *et al.*, 1985). Bile concentrations of erythritol were proportional to plasma erythritol concentrations (Westendorf and Czok, 1983). Erythritol also has been reported to cross the human placenta (Jansson *et al.*, 1993; Schneider *et al.*, 1985) and to pass slowly from the plasma into the brain and cerebrospinal fluid of sheep (Dziegielewska *et al.*, 1979).

In Wistar rats, 0.5 hour after oral dosing with ^{14}C-erythritol at 1.0 g/kg body weight in an aqueous solution, Nakayama (1990c) and Noda *et al.* (1996) reported that 44 and 24% of the administered radioactivity was found in the stomach and small intestine, respectively, with only small amounts detected in the colon. As would be expected, organs involved in the absorption and excretion of erythritol, including the liver, kidney and urinary bladder, also were found to contain relatively higher amounts of radioactivity compared with other tissues. Concentrations in other tissues, except for the cerebellum and cerebrum, were similar to plasma concentrations. In the cerebellum and the cerebrum of rats, erythritol concentrations were around 15% of those reported in the plasma, suggesting that erythritol is not well absorbed across the blood–brain barrier. Over time, erythritol concentrations generally declined in body tissues in parallel with plasma concentrations except in the urinary bladder, and small and large intestines. In these tissues, concentrations remained higher than in plasma, reflecting the rapidity of excretion via the renal route and the transport of unabsorbed erythritol to the large intestine.

Nakayama (1990d) and Noda *et al.* (1996) have reported that the maximum blood concentrations (C_{max}) of erythritol were reached 1 hour after oral dosing of Wistar rats at either 0.125, 0.25, 0.5, 1.0 or 2.0 g/kg body weight. Erythritol concentrations were found to increase in a dose-dependent manner, except for the highest dose level, suggesting saturation of absorption from the small intestine. C_{max} values for each respective dose level were 69, 110, 190, 325 and 469 μg/ml. The distribution ratios of radiolabel in blood cells relative to serum, increased steadily from an average of 18% in blood cells 30 minutes after dosing to 32% and 48% by 1 and 24 hours post-dosing, respectively (Noda *et al.*, 1996). Rapid elimination from the blood occurred at all dose levels, with blood concentrations declining to 2–7% of the C_{max} values within 24 hours of dosing. An initial elimination half-life of 1.7 to 2.0 hours was calculated with dose levels not resulting in saturation of absorption.

The plasma distribution and elimination kinetics of erythritol also have been evaluated in beagle dogs (Nakayama, 1990a; Noda *et al.*, 1996). Following a single oral administration of 1 g ^{14}C-erythritol/kg body weight in an aqueous solution, the concentrations of erythritol in blood and plasma were determined 5, 10, 15 and 30 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, 24, 48, 96 and 120 hours post-dosing. The C_{max} values for erythritol in blood and plasma were found to be 1270 and 1585 μg/ml, respectively, with a T_{max} of 0.5 hour for both. These C_{max} values were somewhat higher than reported for Wistar rats, with the C_{max} attained in a slightly shorter time frame.

In the beagle dog, first compartment elimination half-lives of 0.07 and 0.51 hours were calculated for blood and plasma, respectively (Noda *et al.*, 1996). Elimination from the blood and plasma was rapid, with only 0.9% of the C_{max} (14.6 μg/ml) present in the plasma 24 hours after dosing. A similar concentration remained in the blood. An average of 23% of the radiolabel was found to be distributed to blood cells relative to serum 30 minutes post-dosing. At later times, this value was in the range of 30%.

The effects of erythritol ingestion on its concentrations in plasma and on plasma osmolarity have been measured in different studies with human volunteers. In one study, following an overnight fast, six healthy volunteers received erythritol as a single oral 1 g/kg body weight dose in an aqueous solution (Bornet *et al.*, 1996a). Using an HPLC method, plasma erythritol concentrations were found to increase as early as 10 minutes after oral administration. Maximum plasma concentrations of 1.8 to 2.7 g/litre (14.6 to 22.4 mmol/litre) were attained after 1 to 2 hours, following which the plasma concentrations declined. The average rate of erythritol clearance for the six subjects was reported to be 62 ± 2.8 ml/min. or about 0.5 of the measured

rate for creatinine clearance (120 ± 12.3 ml/min). This rate of clearance is indicative of tubular reabsorption of erythritol by the kidney (Bornet *et al.*, 1996a).

In a second study, four groups of six healthy volunteers each consumed a normal diet which, on a single day, consisted of a controlled breakfast and lunch (Bornet *et al.*, 1996b). Between these two meals, groups consumed a chocolate providing 0.4 or 0.8 g erythritol/kg body weight. One control group received no snack and a second control group received a similar chocolate containing 0.8 g sucrose/kg body weight. Plasma samples were collected at hourly intervals with analysis of erythritol content and plasma osmolarity in addition to other variables. In the group receiving 0.4 g erythritol/kg body weight, plasma concentrations rose to a maximum of 3.0 ± 0.84 mmol/litre at 1 hour post-dosing. Maximum concentrations in the 0.8 g/kg body weight group reached 5.1 ± 0.68 mmol/litre, but occurred at 2 hours post-dosing. Thereafter, plasma concentrations of erythritol declined in both groups, but remained above control values through to the end of the study (i.e. 22 hours post-dosing). At no time were plasma osmolarity values affected by consumption of erythritol at either 0.4 or 0.8 g/kg body weight. Compared with the first study (Bornet *et al.*, 1996a), on a dose-to-dose basis, peak plasma concentrations appeared to be lower after consumption of erythritol in a chocolate snack than in an aqueous solution. This may have resulted from slower gastric emptying associated with the consumption of the chocolate snack compared with the aqueous solution.

Serum concentrations of erythritol also were measured by Noda *et al.* (1994) following the administration of an aqueous solution of erythritol at a dose of 0.3 g/kg body weight to five healthy volunteers who had fasted for a period of 12 hours. This group consumed a lunch 3 hours after dosing with erythritol. A maximum erythritol serum concentration of 426.5 ± 113.4 mg/litre was detected 30 minutes post-dosing. This value declined to 13.5 ± 3.2 mg/litre 24 hours post-dosing. The elimination profile yielded a half-life of 3.4 hours (Noda *et al.*, 1994).

A study with five fasted subjects with non-insulin-dependent diabetes, but who were otherwise healthy and not in need of medication, found, following the consumption of 20 g erythritol in aqueous solution, peak serum concentrations of 649 ± 37.4 mg/litre, with this maximum occurring 1 hour after dosing (Ishikawa *et al.*, 1992, 1996). Following the attainment of the peak serum concentration, concentrations steadily declined so that virtually all erythritol had been eliminated from the serum 24 hours after dosing (Ishikawa *et al.*, 1992, 1996).

Metabolism

The portion of erythritol absorbed during passage through the small intestine may be channelled into normal metabolic pathways of the mammalian organism, provided it is a substrate for the respective enzyme systems, while the unabsorbed portion may be subject to microbial fermentation in the large intestine. For erythritol, an early experiment on the absorption and metabolism of erythritol (of unknown purity) found that respiratory quotient values were not significantly changed after a single 400 mg oral dose to rats (Beck *et al.*, 1938; Carr and Krantz, 1946). Furthermore, in two groups of rats which were fasted for 48 hours and which then received either a fat diet or a fat/erythritol diet (2:1 ratio), low liver glycogen values were found following an 84-hour feeding period. Based on these results, it was concluded that absorbed erythritol was not a substrate for metabolic enzymes and, as a result, was not metabolized to any significant extent and had no gluconeogenic activity (Beck *et al.*, 1938; Carr and Krantz, 1946). Later *in vitro* studies and feeding studies in rodents (Batt *et al.*, 1960a,b; Beck *et al.*, 1938; McCorkindale and Edson, 1954; Noda and Oku, 1990, 1992; Noda *et al.*, 1996; van Ommen *et al.*, 1996), dogs (Noda, 1994) and humans (Bornet *et al.*, 1992; Hiele *et al.*, 1993; Noda *et al.*, 1994) demonstrated that erythritol is not metabolized systemically.

The fraction of erythritol which is not absorbed during passage through the small intestine may be subjected to microbial fermentation in the large intestine. The end-products of this fermentation, mainly short-chain volatile fatty acids such as acetic, propionic and butyric acids, are absorbed and metabolized through established pathways. Gases (CO_2, H_2 and CH_4) also are produced during fermentation. The microbial fermentation of unabsorbed erythritol in the colon is supported by the results of several *in vitro* studies and feeding studies in animals. The fermentation of ^{14}C-erythritol by the caecal microflora of adapted and non-adapted rats was demonstrated in studies conducted by Noda and Oku (1990, 1992). In these studies, ^{14}C-erythritol was found to be degraded at a much higher rate by the microflora of rats which had been adapted to erythritol in the diet for a 2-week period prior to the conduct of the experiment. The main end-products of the fermentation process conducted in a closed anaerobic environment were CO_2 ($>20\%$) and acetic, propionic and butyric acids (approximately 60%). These end-products are similar to the end-products of fermentation of many other carbohydrates which are either poorly absorbed in the small intestine or which are transported to the colon following the ingestion of large doses which saturate the absorptive capacity of the small intestine. The volatile short-chain fatty acids that are produced are rapidly absorbed from the

colon of humans (Dawson *et al.*, 1964; McNeil *et al.*, 1978; Ruppin *et al.*, 1980), dogs (Herschel *et al.*, 1981) and other mammalian species.

Only a small amount (0.1–1.0%) of carbohydrate-derived volatile short-chain fatty acids is lost with the faeces (Florent *et al.*, 1985; Saunders and Wiggins, 1981). Absorbed volatile short-chain fatty acids are efficiently metabolized in mammalian species. Substantial amounts of butyrate, with lesser amounts of propionate, are metabolized in epithelial cells of the colon (Cummings *et al.*, 1987; Vernay, 1987a; von Englehardt and Rechkemmer, 1983). Unmetabolized propionate and much of the absorbed acetate enter the circulation and reach the liver and other tissues. Propionate enters the metabolic pool via methyl-malonyl-CoA and succinate (Vernay, 1987b). Acetate is completely oxidized to CO_2 and water in the citrate cycle (Buckley and Williamson, 1977).

Excretion

Noda and Oku (1992) reported that the proportion of radioactivity excreted as CO_2 increased from 6 to 10% in Wistar rats which had been pre-adapted to erythritol prior to receiving a 0.1 g/kg body weight oral gavage dose of radiolabelled erythritol. The activity of colonic microflora in the excretion of erythritol as CO_2 was further shown by the fact that following intravenous administration, in which there is no exposure of the colonic microflora to erythritol, only about 1% of the administered dose, rather than 6–10%, was excreted as $^{14}CO_2$. Also, germfree rats, which do not have extensive microfloral colonization of the large intestine, have been shown to excrete a reduced percentage of radioactivity in the expired air (0.8% *v.* 6.7 to 10.9%) and an increased percentage in the faeces (13.2% *v.* 6.3 to 6.7%) following oral gavage dosing with 0.1 g radiolabelled erythritol/kg body weight compared with pre-adapted and non-adapted conventional rats (van Ommen and de Bie, 1990; van Ommen *et al.*, 1996). In rats, at increasingly high doses, an increased proportion of the administered dose appears to undergo fermentative metabolism in the colon. This was evidenced by Noda and Oku (1992), who reported that as the single gavage dose of erythritol was increased from 0.01 g/kg body weight to 0.1 and 1.0 g/kg body weight, the proportion of the dose excreted as CO_2 increased from $4.3 \pm 0.6\%$ to $6.0 \pm 1.8\%$ and to $16.2 \pm 3.8\%$, respectively.

Compared with rats, a smaller proportion of orally administered erythritol appears to undergo colonic fermentation in dogs. Following a single oral administration of radiolabelled erythritol at a dose of 1 g/kg body weight, beagle dogs were shown to excrete a cumulative total of $1.17 \pm 0.04\%$ of the dose in expired air within 120 hours of dosing (Nakayama, 1990a; Noda *et al.*, 1996). In the same experiment, rats excreted an average of $4.8 \pm 0.3\%$ of the administered dose in the expired air.

In humans, colonic fermentation of the unabsorbed portion of ingested erythritol is also likely to occur to a small extent. The results of human metabolism studies utilizing high doses of erythritol demonstrate that urinary excretion of unchanged erythritol accounts for up to 90% or more of the administered dose (Bornet *et al.*, 1996a,b; Ishikawa *et al.*, 1992, 1996; Noda *et al.*, 1988, 1994), particularly when urine analyses are extended for a sufficient period of time following dosing as seen in the studies conducted by Noda *et al.* (1988, 1994) and Ishikawa *et al.* (1996). As a result, up to 10% of ingested erythritol is potentially available for microbial fermentation in the large intestine; however, there is evidence from *in vitro* incubation studies of erythritol with colonic microflora from human volunteers (Barry *et al.*, 1992; Hiele *et al.*, 1993) that the colonic microflora of humans do not ferment unabsorbed erythritol to the same extent as rats. Also, at anticipated levels of human consumption of erythritol in foods, only minimal amounts would be expected to remain unabsorbed and subject to fermentation in the large intestine.

Summary

In summary, the available data on erythritol demonstrate it to be rapidly absorbed from the small intestine following oral ingestion and to be excreted virtually quantitatively and unchanged in the urine (Dean *et al.*, 1996; Lina *et al.*, 1996; Nakayama, 1990a,b; Noda and Oku, 1990; Noda *et al.*, 1996; Oku and Noda, 1990a,b; Til *et al.*, 1996; van Ommen and de Bie, 1990; van Ommen *et al.*, 1996). Based on urinary excretion data,

Table 3. Acute LD_{50} values of erythritol in various animal species

Species	Route	LD_{50} (g/kg body weight)	Reference
Rat (m)	iv	6.6	
Rat (f)	iv	9.6	Yamamoto *et al.*, 1987
Rat (m and f)	sc	> 16	Yamamoto *et al.*, 1987
Rat (m)	gav	13.1	Yamamoto *et al.*, 1987
Rat (f)	gav	13.5	Yamamoto *et al.*, 1987
Dog (m)	gav	> 5	Ozeki *et al.*, 1988

iv = intravenous sc = subcutaneous gav = gavage

absorption in animals ranges from 60 to more than 90%. Rats (Oku and Noda, 1990a) and dogs (Dean *et al*., 1996), but not mice (Til *et al*., 1992) were reported to show a dose-dependent decrease in absorption at high oral doses. In humans, as in animals, absorption ranges up to 90% or more (Bornet *et al*., 1996a,b; Ishikawa *et al*., 1992, 1996; Noda *et al*., 1988, 1994), particularly when urine analyses are performed for periods of more than 24 hours following dosing (Ishikawa *et al*., 1996; Noda *et al*., 1988, 1994). Consumption of erythritol with food appears to delay absorption (Bornet *et al*., 1996b). Absorbed erythritol is rapidly distributed throughout the body in both animals and humans, with peak plasma, serum and/or blood concentrations generally occurring within 1 hour of ingestion (Bornet *et al*., 1996a,b; Ishikawa *et al*., 1992, 1996; Nakayama, 1990a,b,c,d; Noda *et al*., 1994, 1996).

The unabsorbed fraction of ingested erythritol, particularly in rats and humans, is potentially subject to microbial fermentation in the large intestine. Fermentation of erythritol in the large intestine produces volatile short-chain fatty acids and gas (Noda and Oku, 1990, 1992). In the rat, at high doses, a higher proportion of the ingested dose undergoes fermentation as the dosage is increased (Noda and Oku, 1992; Oku and Noda, 1990a) and as a result of pre-adaptation to erythritol in the diet (Noda and Oku, 1992). From the available clinical studies, no clear dose-dependent changes in absorption have been observed, mainly because the administered dose was typically 1 g/kg body weight/day or less. In both animals and humans, absorbed erythritol is not subject to systemic metabolism; thus, the absorbed portion does not contribute to the caloric load. Erythritol that is unabsorbed may provide low caloric value through microbial fermentation to volatile short-chain fatty acids in the colon and subsequent systemic absorption and metabolism of these substances.

Toxicological studies

Erythritol has been well studied in a number of toxicological studies. The studies cover the complete spectrum of toxicological investigation, including acute, subchronic, chronic, carcinogenicity, reproductive toxicity, teratogenicity and mutagenicity studies. The results of these studies demonstrate that erythritol is well tolerated, without mutagenic, carcinogenic or teratogenic potential, and is associated only at high doses with physiological effects, including transient laxative effects, slightly decreased weight gains, increased water consumption, increased urine volume, minor changes in urinalysis parameters, and increased caecal and kidney weights. These effects are all physiological, not toxicological, responses which are to be expected given that erythritol is well absorbed, yet rapidly excreted unchanged in the urine and given that at high doses, any unabsorbed erythritol is fermented in the large intestine. Collectively, the results of these studies support the safety of erythritol for use in foods.

Acute studies

The LD_{50} values for erythritol are summarized in Table 3. The results of the acute toxicity studies clearly demonstrate that erythritol has little inherent toxicity since the only effects observed are those commonly associated with the dosing of large volumes of hypertonic solutions. At sufficient doses, such solutions result in cardiac arrhythmia, and osmotic stress-related haemorrhage leading to death. These effects are not specific to erythritol and are of no relevance to potential human exposure to erythritol via the diet. With an oral LD_{50} value in excess of 5 g/kg body weight, erythritol can be classified as essentially non-toxic.

Subchronic studies

Several published (Dean *et al*., 1996; Oku and Noda, 1990a; Til and Modderman, 1996; Til *et al*., 1996) and unpublished (Kamata, 1990a,b; Kanai *et al*., 1992; Shibata *et al*., 1991; Yamaguchi *et al*., 1990; Yamamoto *et al*., 1989) subchronic studies in experimental animals have demonstrated the safety of erythritol for food use and are summarized in Table 4.

28-day studies.

Rats: In a 28-day study (Til and Modderman, 1996; Til and Wijnands, 1991), erythritol was administered to groups of 10 male and 10 female Wistar (Hsd/Cpb:WU) rats as a mixture in the diet at concentrations of 0 (control), 5 or 10%. Food and water were available *ad lib*. throughout the study period. Parameters monitored included clinical observations, body weight, food/water consumption, food utilization efficiency, test substance intake, ophthalmological haematological, clinical chemistry, urinalysis, and organ weights of the adrenals, brain, caecum (full and empty), heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicle, spleen, sublingual salivary glands, submaxillary salivary glands, testes, thymus, thyroid and uterus. Complete gross and histopathological examinations were performed on all major organs and tissues.

No mortality occurred and no changes in appearance or behaviour were reported at any time during the study. Feeding of erythritol to Wistar rats at concentrations of 5 or 10% in the diet for 4 weeks was associated with laxative effects early in the study, slight growth retardation in high-dose males, increased caecal weights in both dose groups and both sexes, significantly increased kidney weights in males with lesser increases in females, and increased serum alkaline phosphatase (ALP) activity in high-dose animals. Other changes in absolute and rela-

Table 4. Summary of the results of subchronic studies performed on erythritol

Species	Sex and number of animals	Route of exposure and dose	Duration	Findings[1]	Reference
Rat (Wistar)	10/sex/dose group	0, 5, or 10% in the diet (approx. 0, 5, or 10 g/kg body weight/day)	28 days	-laxative effects early in the study -slight decrease in body weight gains -increased water consumption -increased cecal and kidney weights -increased serum ALP	Til and Modderman, 1996
Rat (Wistar)	6/sex/dose/treatment group	0, 2, or 5% in the diet to nephrectomized and sham-treated controls (approx. 0, 2, or 5 g/kg body weight/day)	28 days	-increased water consumption	Kanai *et al.*, 1992
Rat (Wistar)	6 males/dose group	0, 1, 5, or 10% in the diet or 10% sorbitol or 10%glycerol in the diet (approx. 0, 1, 5, or 10 g erythritol/kg body weight/day)	28 days	-laxative effects early in the study, of greater severity for sorbitol -increased water consumption -increased urine volume -increased relative caecal weights, more pronounced for sorbitol	Oku and Noda, 1990
Rat (Wistar)	12 females/dose group	0 or 8 g/kg body weight/day by gavage or 8 g/kg body weight/day by gavage with either a high or low electrolyte solution	28 days	-soft stools -increased water intake -increased urine volume and minor changes in urinalysis parameters -increased kidney weights -slight non-significant increase in BUN in erythritol/distilled water group compared with distilled water group -supplementation of water with electrolytes inhibited increase in BUN	Shibata *et al.*, 1991
Rat (Wistar)	15 males/dose group	0, 5, 10, or 20% in the diet, 20% in the diet for 6 hr/day, or 20% mannitol in the diet (approx. 0, 5, 10 or 20 g erythritol/kg body weight/day)	13 weeks	-laxative effects at high doses, also for mannitol -decreased body weight gains -increased food and water intake -increased urine volume and minor changes in urinalysis parameters -increased caecal, relative kidney, and relative bladder weights -increased serum ALP and decreased GGT	Til *et al.*, 1996
Rat (Wistar)	12/sex/dose group	0, 1, 2, 4, or 8 g/kg body weight/day by gavage	13 weeks and 4 weeks recovery	-transient laxative effects -decreased weight gain in high-dose males -increased water intake -increased urine volume and minor changes in urinalysis parameters including electrolytes -increased serum BUN -caecal enlargement and increased kidney weights	Yamamoto *et al.*, 1989
Rat (Wistar)	28/sex/dose group	0, 1, 1.73 or 3 g/kg body weight/day by intravenous injection	6 months and 1 month recovery	-decreased body weight gain -increased water intake -increased urine volume and minor changes in urinalysis parameters -minor changes in haematological parameters -increased kidney weights -increased serum BUN	Kamata, 1990a
Mouse (CD-1)	10/sex/dose group	0, 5, 10 or 20% in the diet (approx. 0, 6, 11 or 23 g/kg body weight/day)	13 weeks	-reduced body weight gain -increased food and water intake -increased urine volume and excretion of electrolytes and enzymes -increased relative caecal and absolute kidney weights	Til *et al.*, 1996
Dog	Three/sex/dose group	0, 1.25, 2.5 or 5.0 g/kg body weight/day by oral gavage	13 weeks and 4 weeks recovery	-various clinical observations -increased urine volume and minor changes in urinalysis parameters -microscopic findings in the kidney typical of functional osmotic diuresis	Yamaguchi *et al.*,
Dog	Six/sex/dose group	0, 1.0, 2.2 or 5.0 g/kg body weight/day by intravenous injection	6 months and 1 month recovery	-various clinical observations -increased water consumption -increased urine volumes, red coloured urine and minor changes in urinalysis parameters -increased BUN	Kamata, 1990b

[1]The reported findings typically occurred at the highest dose tested. For additional details, refer to the description in the text.

tive organ weights and in haematological, clinical chemistry and urinalysis parameters were generally of a small magnitude, often were not dose related, occurred in one sex only, and were usually within the physiological range, and, therefore, were not considered to be treatment related or biologically significant.

The observed slight decrease in body weight gain in the high-dose males was probably the result of the reduced caloric content of the erythritol-containing diet. The increased caecal weights, both full and empty, could be ascribed to the presence of increased amounts of osmotically active and fermentable substances (i.e. erythritol and short-chain fatty acids resulting from microbial fermentation of any erythritol which was not absorbed in the small intestine) in the large intestine (Newberne *et al*., 1988) which is known to occur at these doses based on the results of metabolic studies (Noda and Oku, 1990, 1992; Oku and Noda, 1990a). The exact mechanism by which increased loading of the large intestine with osmotically active or fermentable substances results in caecal enlargement in rodents is not directly known. However, this is a common finding in rodents administered carbohydrates such as raw potato starch, chemically modified starches, lactose, polyols, pectins and alginates that are fermented in the large intestine (Smits-van Prooije *et al*., 1990). Increased caecal weights may be the result of the trophic effect on the colonic mucosa associated with the reduction in faecal pH caused by the presence of fermentation products (e.g. volatile short-chain fatty acids) (Sakata, 1987). Increased faecal bulk, probably resulting from an increase in water content in response to the presence of osmotically active substances (Leegwater *et al*., 1974; Walker, 1978), also may play a role. In any case, the increased caecal weights were considered a physiological response of no toxicological significance (WHO, 1987) since no histopathological correlates were found (Til and Modderman, 1996). The reported increased serum ALP activity was also thought to be related to caecal enlargement (Til and Modderman, 1996) since the intestine is considered to be the main source of this enzyme in non-fasting rats (Righetti and Kaplan, 1971). Moreover, other substances which produce an increased osmotic load in the large intestine, including slowly digested carbohydrates, have been shown to increase ALP in association with caecal enlargement (Bär *et al*., 1995; Moser *et al*., 1980; Schaafsma and Visser, 1980; Woutersen, 1987).

The reported increase in kidney weights in the males was not accompanied by impaired renal function as measured by standard urinalysis parameters or by gross and histopathological examinations (Til and Modderman, 1996). The rapid absorption and excretion of erythritol are known to induce a diuretic effect at the dose levels used in the study, resulting in an increased workload on the kidneys. Diuresis has been demonstrated in rodents to result in increased kidney weights (Bär *et al*., 1995; Ogino *et al*., 1994; Sterck *et al*., 1992). Based on these considerations, the increased kidney weights in this study were considered to be a physiological response rather than a toxicological effect. The diuretic effect of erythritol was also evidenced by the significantly increased water consumption in the high-dose animals. Til and Modderman (1996) concluded that the feeding of erythritol at a dietary level of 10% did not result in overt signs of toxicity.

Another 28-day toxicity study was specifically designed to assess the potential effects of erythritol on renal function (Kanai *et al*., 1992). In this study, erythritol (99.9% purity) was administered in the diet at concentrations of 0, 2 or 5% to groups of six male Wistar (Slc) rats. Prior to dosing, six rats/dose group were subjected to nephrectomy resulting in loss of about 70% of renal function, while six rats/dose group served as sham-operated controls. Animals were observed daily for signs of ill-health and were regularly monitored for body weight gain, food/water consumption and erythritol intake. Ophthalmological, haematological, clinical chemistry and urinalysis parameters were measured and organ weight determinations made for the brain, pituitary, thyroid glands, parathyroid glands, salivary glands, thymus, lungs, bronchi, heart, liver, spleen, adrenals, kidneys, testes, seminal vesicles and prostate at necropsy. Gross pathological examinations were conducted and samples of major tissue collected.

The feeding of erythritol to nephrectomized and sham-treated Wistar rats at concentrations of 2 or 5% in the diet for 4 weeks had no significant effects on mortality, clinical signs, body weights or food consumption. In contrast to the results of the Til and Modderman (1996) study, no laxative effects were recorded by Kanai *et al*. (1992), possibly due to their use of 5% as the maximum dose level compared with 10% in the Til and Modderman (1996) study. Increased water consumption in the 5% dose group of the sham-treated and nephrectomized rats compared with controls was considered to be evidence of a diuretic effect of erythritol. No toxicologically significant effects on haematological, clinical chemistry or urinalysis parameters were associated with erythritol treatment. Although several statistically significant changes in these parameters were reported, any changes were found to either be of a small order of magnitude, to lack a dose–response effect, to occur in one sex only, or to lie within the normal physiological range. Nephrectomized rats fed erythritol-containing or control diets both exhibited the expected physiological changes in haematological and urinary parameters. Organ weights were not affected by erythritol treatment, although caecal weights were not recorded. No significant differences were found between controls and ery-

Table 5. Summary of clinical studies on erythritol

Study objective	Administration protocol	Population	Reference
Estimate threshold for laxation	single (oral) dose in food (jelly)	healthy male and female volunteers	Oku and Okazaki, 1996a,b
Estimate threshold for laxation	single oral dose in solution	healthy male and female volunteers	Takahashi, 1992b (unpublished)
Estimate threshold for laxation	single oral dose in solution	healthy male volunteers	Umeki, 1992 (unpublished)
Study urinary excretion	single oral dose in solution	healthy male volunteers	Noda *et al.*, 1988 (unpublished)
Study plasma and urine kinetics	single oral dose in solution	healthy male and female volunteers	Bornet *et al.*, 1996a (published)
Evaluate gastrointestinal response and study plasma and urine kinetics	single (oral) dose in food (chocolate)	healthy male and female volunteers	Bornet et al, 1996b (published)
Evaluate laxation after continuous dosing	5-day oral ingestion in solution	healthy male and female volunteers	Takahashi, 1992a (unpublished)
Evaluate tolerance to subchronic ingestion	14-day, double-blind, 2-way cross-over study with sucrose	healthy male volunteers	Tetzloff, 1996 (unpublished); Tetzloff *et al.*, 1996 (published)
Evaluate effects on carbohydrate metabolism and on urinary excretion	single oral dose in solution	healthy male volunteers	Noda *et al.*, 1994 (published)
Evaluate effects on carbohydrate metabolism in diabetics	single oral dose in solution	healthy diabetics	Ishikawa *et al.*, 1992 (unpublished); Ishikawa *et al.*, 1996
Evaluate effects on carbohydrate metabolism and on renal function in diabetics	14-day continuous (oral) dosing in solution	healthy diabetics	Miyashita *et al.*, 1993 (unpublished); Ishikawa *et al.*, 1996 (published)

thritol-treated groups at gross and histopathological examination.

On the basis of the results of the study, it was concluded that diets containing 2 to 5% erythritol do not cause any worsening of renal impairment associated with partial nephrectomy. Moreover, erythritol did not cause any toxicologically significant effects in healthy sham-treated rats. The results of the Kanai *et al.* (1992) study support the conclusion of Til and Modderman (1996) that increased kidney weights, in the absence of histopathological or functional changes, are a physiological response to the diuretic properties of high-dose administration of erythritol.

Similar results have been reported in a combined metabolic/toxicity study (Oku and Noda, 1990a). In this study, groups of six male Wistar rats received erythritol mixed in the diet at a concentration of 0, 1, 5 or 10% or received a diet containing 10% sorbitol or 10% glycerol. All rats were allowed to eat and drink *ad lib*. During the study, clinical signs were monitored, body weights and food/water consumption, test substance intake and urine volume were recorded every 4 days, clinical chemistry (total protein, glucose, cholesterol and triacylgylcerol) values were determined at necropsy, and organ weight determinations were made at terminal necropsy for the liver, kidneys, small intestine, caecum and colon. No pathological examinations were conducted. Effects of treatment on intestinal digestive enzyme activity, including sucrose, maltase, isomaltase, ALP and leucine aminopeptidase (LAP-C) were determined for all rats at study termination.

Overall, feeding of erythritol to Wistar rats at concentrations of 10% in the diet for 28 days was not associated with mortality or effects on body weight, food intake or food utilization efficiency. In the rats receiving erythritol at 10% in the diet, laxative effects were observed early in the study, but resolved later, suggesting microbial adaptation to the entry of unabsorbed erythritol into the large intestine. At the same dose, however, sorbitol caused much more severe laxative effects, probably due to its slower rate of absorption and hence likely greater amounts reaching the lower gut. Laxation associated with entry of large quantities of low molecular weight substances into the large intestine has been widely reported and is thought to be due to decreased transit times associated with the increased osmolarity of the colonic content. The observation of increased relative caecal weights in the high-dose erythritol-treated animals similarly was considered to be a physiological response resulting from the increased osmotic load to the colon. As might be expected, sorbitol, which is less well absorbed and reaches the lower gut in higher concentrations than erythritol, produced a greater increase in relative caecal weights compared with the same dose of erythritol.

Erythritol was demonstrated to produce diuretic effects marked by an increased urine volume, particularly at the 10% dose level. This effect was considered to be the result of a likely increase in the osmolarity of the blood resulting from the high rates of absorption and lack of metabolism of erythritol. Diuresis also explains the observation of increased water consumption rates in the erythritol-treated rats. Clinical chemistry measurements revealed a slight decrease in serum triacylgylcerol concentrations. This observation was considered not to be of toxicological significance. The results of the intestinal enzyme assays showed no significant effect of erythritol or glycerol treatment. Sucrose, isomaltase, ALP and LAP-C were significantly decreased in the sorbitol-treated rats.

The results of 13-week studies in rats have essentially extended and confirmed the observations and conclusions reported in the 28-day studies in demonstrating that high doses of erythritol are well tolerated and are only associated with physiological responses brought about by diuresis and the presence of increased quantities of osmotically active, fermentable substances in the large intestine (Newberne *et al*., 1988).

13-week studies.

Rats: An exploratory 13-week study in rats, which compared the effects of consumption of erythritol in the diet with the effects of mannitol and evaluated the impact of food restriction on these effects, was conducted (Til *et al*., 1991, 1996). In this study, groups of 15 male Wistar rats were fed diets containing erythritol at levels of 0 (control), 5, 10 or 20%. One other group of 15 rats received a diet containing 20% mannitol while another similar group received a diet containing 20% erythritol, but which was only available for 6 hours/day. Rats were observed for signs of ill-health, and measurements were made for food and water consumption, standard haematological, clinical chemistry and urinalysis parameters including analysis of γ-glutamyl transferase (GGT) and *N*-acetyl glucosaminidase (NAG), urinalysis parameters which were not measured in the 28-day studies. Organ weight determinations were made at terminal necropsy on all key organs including the caecum and kidneys. Also, a complete gross and histopathological examination was performed on all rats. In addition, faecal and urine samples were analysed for the presence of mannitol or erythritol.

As a result of treatment, loose stools were observed occasionally in rats fed diets containing 20% erythritol or 20% mannitol, with the degree of this effect greater in the mannitol-treated group. Body weights were statistically reduced in the 20% erythritol and 20% mannitol groups throughout the study, with the greatest effect seen in the 20% restricted erythritol group. Body weights in the 10% erythritol group were slightly reduced compared with controls. Food intake was increased over controls in all erythritol groups in a dose-related manner. The reduced body weight gain in the highest dose erythritol group despite higher food intakes was attributed to the lower caloric content of erythritol and represents a physiological response, contributed to by the reduced intestinal transit time associated with higher erythritol doses. Water intake was statistically increased in the 10% and both 20% erythritol groups and was slightly increased in the 5% erythritol and 20% mannitol groups.

Reported minor haematological changes were considered not to be treatment related based on the lack of dose–response, occurrence in one sex or at one time point only, and the small magnitude of the changes involved. Clinical chemistry analysis revealed an increased plasma alkaline phosphatase activity in the 20% restricted erythritol group and in the 20% mannitol group, slightly decreased plasma GGT activity in the 20% erythritol and mannitol groups, and slightly increased plasma inorganic phosphate concentrations in the 20% restricted erythritol group. Increased levels of serum alkaline phosphatase activity were reported previously in a 28-day study (Til and Modderman, 1996), and may have been the result of increased release from the intestinal mucosa associated with the presence of increased amounts of osmotically active substances in the gastrointestinal tract and decreased transit times.

Urine production was increased in direct relation to the erythritol dose and was accompanied by lower osmolality. Urine pH was slightly increased in all erythritol groups and in the 20% mannitol group at week 10. Increased urine output was attributed to the increased load on the kidneys by the high renal clearance rate of erythritol. This was also evidenced by the increased water intake. Accordingly, urinary outputs of electrolytes were not changed absolutely, but concentrations were lower. Calcium excretion was increased in the 20% erythritol group and was attributed to enhanced calcium absorption from the gut, an effect seen with other osmotically active substances which reach the large intestine at high concentrations (Bär, 1985; Dills, 1989; Roe, 1989; Senti, 1986). Creatinine-normalized excretion of GGT was increased in the restricted 20% erythritol group, probably as a result of decreased creatinine excretion in this group as opposed to an increased enzyme excretion. The urinary excretion of NAG was found to be slightly increased in both 20% erythritol-fed groups. Given the lack of histopathological effects in the kidneys, this increase was considered to be due to osmotic diuresis (polyuria) brought about by the rapid absorption of erythritol. NAG, a lysosomal enzyme of renal tubular cells, and GGT, a brush-border enzyme, are known to be increased under conditions of increased urinary flow (Burchardt and

Jung, 1992), and, as such, represent a physiological response rather than a toxicologically significant effect.

As reported in the 28-day studies on erythritol, statistically increased absolute and relative weights of the caecum, both full and empty, were noted by Til *et al.* (1996) for the 10 and 20% erythritol and 20% mannitol groups, with the mannitol group showing the greatest increases. The much greater amount of mannitol passing through to the large intestine, due to its lower rate of absorption, explains the greater caecal enlargement (full and empty), reduced faecal pH, and soft or loose stools seen in this group compared with the erythritol-treated groups. The absence of pathological changes in the caecum indicates that these changes were of no toxicological significance. The relative kidney weights exhibited a dose-dependent increase in the 10 and 20% erythritol groups. Relative bladder weights were also slightly increased in the food restricted 20% erythritol group. The increased relative kidney weights were thought to be due to the increased workload on the kidneys brought about by the diuretic effects of high doses of erythritol. The increased relative bladder weights in the restricted 20% erythritol groups also may have been associated with polyuria in combination with greater reduction in body weight seen in this group. Other reported changes in organ weight parameters were considered to be of no toxicological significance since they were not accompanied by histological change and were generally related to decreased body weights.

Overall, no significant toxicity was attributed to the test article although some physiological responses, including decreased weight gains, increased urinary volumes, minor changes in urinalysis parameters, and increased caecal and kidney weights were observed.

Another 13-week study (Yamamoto *et al.*, 1989), which included a 4-week recovery period, also showed similar results. In this study, erythritol (99.7% purity) was administered by oral gavage as an aqueous solution to groups of 12 Slc:Wistar rats of each sex at doses of 0, 1, 2, 4 or 8 g/kg body weight/day. An additional six rats/sex/dose were administered erythritol at 0 (control), 4 and 8 g/kg body weight/day for 13 weeks and allowed to recover for 4 weeks. Variables measured included observations for ill-health, body weight, food consumption, water consumption, sensory response, ophthalmological, haematological, clinical chemistry, urinalysis, determinations of key organs, but not including the caecum, and gross and microscopic pathology of all major organs and tissues. Electron microscopic examinations were performed on the liver and kidney of two animals of each sex from the control, 1, 4 and 8 g/kg body weight/day groups and from two animals of each sex in the control, 4 and 8 g/kg body weight/day recovery groups.

There were no treatment-related mortalities or effects on sensory function, ophthalmological findings or haematological parameters. Soft or loose stools occurred early in animals treated at 4 or 8 g/kg body weight/day, and occurred sporadically thereafter, probably as a result of increased water content of the faeces due to osmotic changes, increased motility, and increased intestinal microvascular permeability, all of which are effects common to osmotically active substances that reach the large intestine. Body weights were statistically reduced in the high-dose males from week 7 up to the first week of the recovery period, after which they were comparable to controls. No differences were observed in the other treatment groups. The reduced weight gains in the high-dose males were considered to be due to the transient laxative effects of erythritol at this dose as well as to the reduced food intake during the dosing period. Water intake was statistically increased in the high-dose animals at most time points during the treatment period, but was comparable to controls during the recovery period. No effects on food/water intake occurred in the 1, 2 or 4 g/kg body weight/day groups at any time.

Effects of erythritol treatment on urinalysis parameters included increased urine volume in males and females in the high-dose group, increased specific gravity and osmotic pressure for the 1, 2 and 4 g/kg body weight/day males, increased sodium and chloride excretion/18 hours in males and females in the two highest dose groups, and increased potassium in high-dose animals. No changes in urinalysis parameters were seen at the end of the recovery period. Urinary changes, including the increases in specific gravity and osmotic pressure, were considered to have been related to the increased load on the kidneys produced by the large amount of erythritol excreted in the urine. This was evidenced by the reversal of effects during the recovery period. At low doses, this translated into higher specific gravity and osmotic pressure whereas at the higher doses, lower values were seen. Increased water intake, urine volume and increased urine electrolyte excretion were due to the diuretic effects of erythritol. Corresponding changes in serum electrolyte values were observed only for sodium and potassium and reflected diuresis, producing only slight differences relative to normal values. Reported increases in serum blood urea nitrogen (BUN) in the 4 g/kg body weight/day females and both sexes of rats at the high dose were considered to be due to diuresis/laxation and associated increased loss of electrolytes. Other causes known to be associated with increased BUN values (i.e. renal failure, excessive intake of protein, or lysis of tissues) were not apparent. Finally, a reported decrease in serum alpha-1 globulin in

high-dose animals was attributed to diuresis since less reabsorption in the kidney would occur.

As with other studies conducted on erythritol, caecal enlargement occurred at the highest dose level and was considered to have been due to fermentation of erythritol by enterobacteria in the caecum. Other factors, such as osmotic water loading of the large intestine, were also important.

In the high-dose group, increased kidney weights and slight swelling of the kidney were reported, and as discussed for other studies, were considered to have been due to diuresis. Microscopically, slight dilation, without obstruction, of the renal tubules was observed in the 8 g/kg body weight/day males and females. Electron microscopic examination showed tubular epithelial cell and lumen dilation, without cytoplasmic microstructure changes. All of these observations were consistent with a diuretic effect of erythritol. Calcification of the kidneys occurred but was considered not to have been due to treatment. No changes were observed at the end of the recovery period.

In conclusion, all the effects reported in the Yamamoto *et al.* (1989) study appeared to be due to the osmotic changes (caecal and renal) associated with erythritol. No overt toxicity was observed and all reported changes, including the reported increase in serum BUN, were considered to represent physiological responses to the diuretic and osmotic properties of erythritol.

To further evaluate the finding of increased BUN in the 13-week Yamamoto *et al.* (1989) study and to assess the suspected role of increased electrolyte excretion, Shibata *et al.* (1991) performed a supplementary 1-month-long study in which erythritol was administered to groups of 12 female Wistar rats by gavage in distilled water at doses of 0 (control) or 8 g/kg body weight/day. Two additional groups were given 8 g erythritol/kg body weight/day and drinking water containing either a low electrolyte solution (Na^+ 35 mmol/litre, K^+ 35 mmol/litre, Cl^- 80 mmol/litre, Ca^{2+} 5 mmol/litre) or a high electrolyte solution (Na^+ 70 mmol/litre, K^+ 70 mmol/litre, Cl^- 160 mmol/litre, Ca^{2+} 10 mmol/litre). Control groups received the drinking water with low or high electrolyte solutions. Observations and analyses conducted were similar to the previously described 13-week study (Yamamoto *et al.*, 1989), except for a reduced number of haematological, clinical chemistry (but still including BUN values) and urinary parameters and determination of kidney weights only. Also, following the gross examinations, only the kidneys were subject to histopathological and electron microscopic examinations.

As expected for an osmotically active substance administered at doses high enough to result in transport to the large intestine, soft stools were seen in all groups fed erythritol, an effect which was not altered by the different electrolyte administrations (Shibata *et al.*, 1991). Food intake showed an initial decrease (days 1 to 3) in the erythritol/tap water group and for several time periods in the other erythritol groups. Also, food intake compared with the erythritol/tap water group was decreased in both erythritol/electrolyte groups. These changes were not considered to be due to erythritol, but the result of the intake of hypertonic solutions since the food intake decreases in the erythritol/tap water group were only seen at the beginning of the study. The changes in water intake parallelled those of urinary volume and the occurrence of loose stools in the erythritol-treated groups. These effects were attributed to the osmotic/diuretic effects of erythritol.

Increased BUN at 8 g/kg body weight reported by Yamamoto *et al.* (1989) was not replicated in this study, since no statistical differences were observed for serum BUN in the erythritol and erythritol/low electrolyte groups. In the erythritol/high electrolyte group, a statistical decrease was reported. These changes were considered to be the result of differences in electrolyte intake, particularly sodium, in these groups. As a result, without sodium supplementation, BUN would tend to be increased by erythritol and its associated diuretic effects, as was seen to a slight degree in the erythritol and erythritol/low electrolyte groups in this study and in the 13-week study (Yamamoto *et al.*, 1989). BUN increases associated with diuresis and electrolyte loss have been reported for the diuretic muzolimine (Garthoff *et al.*, 1982) and have been shown to occur in rats fed hyposodium diets (Wassner, 1989). Reported alterations in serum and urinary magnesium and protein, and urinary osmotic pressure, also are associated with the diuretic effect of erythritol (Shibata *et al.*, 1991).

The kidney weights in the erythritol-treated groups were increased as expected and were considered to reflect the increased work load resulting from diuresis since no significant pathological changes were present and there was no indication that renal function had been altered.

Mice: Effects similar to those reported in rats (Til *et al.*, 1996) have been reported in a 13-week mouse study (Til *et al.*, 1992, 1996). 10 male and 10 female CD-1 mice were fed erythritol mixed in the diet at concentrations of 0, 5, 10 or 20%. As a result of treatment, there were no effects on mortality or clinical signs. As seen in the rat studies, body weights were statistically reduced in the high-dose males, with lesser reductions also noted in the mid- and high-dose females and low- and mid-dose males. Compared with the controls, food intake appeared higher in dosed females and high-dose males. The reduced body weight gain in the highest dose group, despite a higher food intake, was attributed to the lower caloric content of the erythritol-containing diet, contributed to by the reduced intestinal transit time associated with higher erythritol doses. The reduced body weight

gains of both sexes treated at the 10% dose level and in the males at the 5% dose level similarly were probably due to the reduced caloric content of the diets ingested.

Water intake was significantly increased in the 20% dose groups at all time points measured, in the mid-dose males at all time points, in the mid-dose females at week 4, and in the low-dose animals only on a few days. There were no clinically significant effects of erythritol treatment on haematological or blood chemistry parameters. Observed slight, but significant, increases in relative caecal weights in the high-dose animals were ascribed to the presence of increased amounts of osmotically active and fermentable substances (i.e. erythritol and short-chain fatty acids resulting from microbial fermentation of any erythritol which was not absorbed in the small intestine) in the large intestine.

Reported increases in absolute and relative kidney weights in the high-dose animals and increases in the relative kidney weights of the low- and mid-dose males were not accompanied by impaired renal function as measured by urinalysis parameters or by gross or histopathological examinations. As previously discussed, the kidney weight increases were ascribed to the rapid absorption and excretion of erythritol which is known to induce a diuretic effect, resulting in an increased workload on the kidneys. This was evidenced by the marked increase in urine volume, decrease in osmolality at the high-dose, and increased water intakes. An observed increase in calcium excretion/24 hours in the high-dose group was speculated to be the result of increased calcium absorption, an effect of fermentation in the large intestine reported for other poorly absorbed or poorly digested low molecular weight compounds. The increases in calcium excretion, however, were not accompanied by nephrocalcinosis in the renal medulla or pelvis as has been observed with certain polyols (Conz and Maraschin, 1992; Dills, 1989; Senti, 1986; Sinkeldam *et al.*, 1992; Woutersen, 1987). The lack of histopathological changes in the renal tissues suggests that observed increases in excretion of protein, creatinine and GGT and NAG activity in the high-dose animals were not indicative of renal damage (Til *et al.*, 1992, 1996). Excretion of NAG was unchanged on a creatinine-normalized basis. At the 10% dose level, creatinine-normalized urinary excretion of protein was increased in both sexes, with excretion of GGT increased in males only. Other increases in electrolyte excretion, including sodium, potassium and phosphate, also were considered to be due to marked osmotic diuresis. The loss of potassium in the urine did not result in hypokalaemia since the potassium concentration in the plasma remained stable. The expected effects on urinalysis parameters essentially parallelled those seen in rats.

Overall, Til *et al.* (1992, 1996) attributed no significant toxicity to erythritol although some physiological responses, as might be expected given the physical-chemical properties and metabolic profile of erythritol, including reduced body weight gain, increased urinary volumes, increased electrolyte and urinary enzyme excretion, increased kidney weights, and caecal enlargement, occurred.

Dogs: One 13-week study evaluated the effects of erythritol in a non-rodent species (Yamaguchi *et al.*, 1990). In this study, erythritol was administered by gavage as an aqueous solution to groups of three beagle dogs of each sex at doses of 0, 1.25, 2.5 or 5.0 g/kg body weight/day. An additional two dogs/sex were added in the control, mid- and high-dose groups to assess the effect of a 4-week recovery period. Observation included signs of ill-health, and body weight change, food consumption and water consumption rates. Standard ophthalmological examinations, haematological, clinical chemistry, urinalysis and faecal analysis parameters were measured throughout the study. In addition, electrocardiograms and liver and renal function tests were performed. At necropsy, all animals were examined grossly, organ weights were determined, and all major tissues were subject to histological examination. As a result of treatment, there were no mortalities or clinically significant effects on body weight gain, food consumption rates, ophthalmological examinations, electrocardiogram and liver and renal function tests, or on haematological or blood chemistry parameters.

Erythritol treatment of beagle dogs was associated with several changes in general condition, including dryness/redness of the mucosal membranes, epidermal redness, salivation, vomiting and soft or loose stools. In general, the time to occurrence and duration of these effects were dose related but disappeared within 24 hours of dosing. As a result, these changes were considered by Yamaguchi *et al.* (1990) to have been caused by increased plasma osmotic pressure resulting in an influx of interstitial and intracellular fluid into blood vessels, increasing blood volume and diuresis, producing dryness and redness of mucosal and nasal membranes and the epidermis. Salivation, vomiting and soft faeces were considered to be due to an upset of the gastrointestinal water balance by erythritol, similar to the above systemic water changes. The disappearance of effects during the recovery period and the lack of changes in weight gain or food intake suggest that the above changes were not toxicological, but physiologically based.

Changes in urinary parameters (i.e. increased volume in high-dose groups, increased specific gravity in females, increased osmolarity in low- and mid-dose females, decreased potassium excretion in mid- and high-dose males, and light coloration in all treatment groups), as well as increased water consumption in treated animals, were considered to have been due to the diuretic effects of erythritol and the excretion of erythritol in the urine. In this

study, the degree of any diuresis, however, was not excessive since no changes in urinary sodium or chloride excretion were observed. Further, serum biochemical parameters were not affected. Also, all changes were reversed during the recovery period.

In treated dogs, there were indications to irritation of gastrointestinal mucosa, as evidenced by the appearance of occult blood in conjunction with loose stools, and one dog in the high-dose females with jelly-like, occult blood-containing stool. Redness of the intestinal mucous membrane was observed in the higher dose groups. These effects also were attributed to the increased water load and faecal movement induced by erythritol.

Reported changes in thymus weight and thymic atrophy noted in some mid- and high-dose dogs were not accompanied by changes in blood or biochemical parameters which would suggest interference with immunological function. Consequently, non-specific stress caused by continuous treatment was considered the cause of this effect. Other reported changes in organ weights were considered to be of no clinical significance.

Microscopic findings in the kidney (i.e. eosinophilic degeneration, slight tubular dilatation, pycnosis, hydropic degeneration and slight necrosis) of a few high-dose male dogs were reported to be typical of a transient functional osmotic nephrosis response induced by treatment with highly osmotic solutions. As a result, these changes were considered not to have been specific to erythritol but a general response of osmotic nephrosis. No significant renal findings were reported at the end of the recovery period nor were there any indications of impaired renal function, as shown by blood biochemistry, urinalysis or renal function tests. Yamaguchi *et al.* (1990) concluded that the effects seen in their study were non-specific in nature and related to the general diuretic and osmotic actions of erythritol.

In addition to the numerous 28-day and 13-week oral studies conducted in rats, mice and dogs, two 6-month intravenous administration studies, one in rats (Kamata, 1990a) and one in dogs (Kamata, 1990b), have been performed. The results of these studies provide further evidence to demonstrate that observations reported in the oral and gavage studies are the result of physiological responses to high doses of erythritol.

6-month studies.

Rats: In the 6-month intravenous administration study in rats (Kamata, 1990a), groups of 28 Slc:Wistar rats of each sex were administered erythritol by intravenous injection in a distilled water vehicle at doses of 0 (control), 1, 1.73 or 3 g/kg body weight/day. Controls received injections of physiological saline. Six rats/sex/group in the control, mid- and high-dose groups were observed for an additional 4 weeks after treatment.

No effects of erythritol treatment by intravenous injection were observed on mortality, clinical signs, or on the results of the ophthalmological, gross pathological and histopathological examinations. Body weights were statistically reduced in the 3 g/kg body weight/day males from week 8 to the end of the recovery period, but were increased in the 1 g/kg body weight/day males on days 168 and 175. The high-dose females showed significantly decreased body weights on days 126 of treatment and on day 2 of the recovery period, but final body weights, both at the end of the treatment and recovery periods, were comparable to controls.

Increased water intake, urinary volume and frequent urination were noted in treated animals, particularly at the high-dose level. These effects were ascribed to the diuretic properties of the injected erythritol solution. As a consequence of these changes, there was a reduced concentration of urinary protein at the higher dose levels. Observed reductions in urinary excretion of electrolytes, rather than increased excretion seen in many other studies, and the reported reduction in urine pH, in the treated animals were considered by Kamata (1990a) to have been due to the injection of controls with physiological saline as opposed to distilled water which served as the vehicle for the treated animals. In the treated animals, the reduced loading of electrolytes due to the use of distilled water would result in the appearance of a comparatively decreased urinary excretion of these electrolytes.

The results of the haematological examinations revealed dose-related decreased RBC, haematocrit, and haemoglobin in all treated male groups and in the top two dose groups in females. Reticulocyte counts were statistically increased in the high-dose males and in mid- and high-dose females. Activated partial thromboplastin time (APTT) also was decreased in all treated female groups. No alterations in haematological parameters were observed in any group at the end of the recovery period. As with the urinary parameters, the use of physiological saline as the control injection was considered to be the likely explanation for the reduced RBC count, haematocrit and haemoglobin, increased reticulocyte counts, and the reduction in APTT. Kamata (1990a) reported that similar changes have been seen with the injection of distilled water or highly osmotic solutions.

Clinical chemistry analysis revealed a number of changes; however, most were considered to be of no biological significance due to their small magnitude, reversal in recovery period, or distribution with the normal physiological range. In addition, none of the blood chemistry parameter changes was accompanied by pathological changes. An increase in BUN was observed in the high-dose females. This effect which also had been reported in 13-week gavage (Yamamoto *et al.*, 1989) and 28-day gavage studies (Shibata *et al.*, 1991), appeared to be associ-

ated with the diuretic action of erythritol. As there were no indications of renal impairment (i.e. creatinine was not altered and no renal pathology was noted) in these studies, the reported slight increases in serum BUN were considered to be of no clinical significance.

Among the various organ weight differences seen between the treated and control groups, most were considered secondary to the reduced body weights seen at the upper dose levels (Kamata, 1990a). Kidney weight increases reported in the high-dose animals were considered the result of an increased load on the kidney in response to the excretion of erythritol. The observation of increased adrenal weights in high-dose animals and of decreased thymus weights in high-dose males may be a physiological response to high-dose intravenous administration of erythritol. No pathological changes accompanied any of the organ weight changes. As expected, no increases in caecal weights were reported. This is in keeping with the known mode of action of erythritol, since administration of erythritol by intravenous injection would not lead to an increased amount of osmotically active substances in the large intestine. Similarly, in this study were no reports of any laxative effects associated with erythritol treatment.

Dogs: A second 6-month intravenous injection study has also been carried out in dogs (Kamata, 1990b). Erythritol was administered by intravenous injection to groups of six male and six female beagle dogs at doses of 0, 1.0, 2.2 or 5.0 g/kg body weight/day. Two additional females in the control and high-dose animals were added later in a supplementary study. Also, to investigate the reversibility of any effects, four dogs/sex in the control, mid- and high-dose groups were observed for an additional 4 weeks after treatment. In addition to the standard observations, electrocardiogram and liver and renal function tests were performed.

Effects attributable to intravenous injection of erythritol included a generally increased frequency of urination and vomiting, hypoactivity, and the appearance of red-coloured urine early in the study, particularly in the higher dose groups. As seen in rats dosed by intravenous administration, no increase in the incidence of soft stools or other signs of laxation occurred in treated dogs at any time during the study. Water consumption was also increased in both sexes throughout the treatment period. There were no effects of treatment on body weight gain. Two high-dose females died as the result of over-dosing. These animals were replaced with two supplementary animals.

The results of the haematological examinations failed to detect any clear treatment-related effects. Serum potassium levels appeared to be increased in the mid- and high-dose females and high-dose males. From the biochemical analyses, there appeared to be a treatment-related increase in BUN. Often BUN is used as a marker to detect renal damage. However, in this study, the BUN levels were within the normal range of physiological variation, there were no histopathological changes noted in the kidney, and there was no concomitant increase in creatinine concentrations to indicate the presence of renal damage. Also, the results of the renal function tests did not reveal any evidence of renal function impairment. As a result, the increase in BUN was considered to be of no toxicological significance.

Urinalysis revealed that at 3 and 6 months of treatment, urine volumes were increased and specific gravity decreased in all but the low-dose females. All these effects were considered to be the result of the diuretic action of erythritol, and not toxicological effects. No effects on urinalysis parameters were apparent following the 4-week recovery period. The red-coloured urine was thought to be due to distension of the bladder by the increased urine volumes and subsequent rupture of fragile capillaries located in the bladder mucosa. This effect, however, was transitory as evidenced by the lack of histopathological change at necropsy. Other reported changes in urinary electrolytes, particularly lower sodium and chloride levels in all treated groups, were attributed to relatively high values of the control group which probably resulted from the treatment of the controls with physiological saline instead of distilled water which was used as the vehicle for the erythritol-treated groups. Other changes noted in urinalysis parameters were either transitory in nature, showed no dose–response or consistency across sex, and thus were considered to be chance findings of no toxicological significance.

Including the supplemental animals which were placed on study to replace two decedent high-dose females, at the end of the treatment period, a number of statistically significant differences in organ weight parameters were recorded, including increased absolute brain weight in the mid-dose males, decreased absolute left kidney and left sublingual gland weights in the low-dose males, and decreased relative spleen weights in the low-dose females. At the end of the recovery period, increased absolute right kidney weights in the mid-dose males and increased relative lung and liver weights were recorded for the high-dose males. Decreased absolute weights of the cerebrum, left thyroid, left kidney and right submandibular gland were observed in the mid-dose females. Owing to the small magnitude, lack of consistency across sex and dose, and given the lack of any histopathological correlates, the absolute and relative organ weight changes were considered not to be of biological significance.

Kamata (1990b) concluded that all treatment-related effects of intravenously injected erythritol in the 6-month dog study appeared to be due to the osmotic changes. No overt toxicity was observed,

and all the treatment-related effects were reversible on the cessation of exposure, a hallmark indication that these effects were due to physiological responses, not toxicity.

Chronic studies. The potential chronic toxicity of erythritol was investigated in a dog study and two long-term rat studies (Dean *et al*., 1996; Lina *et al*., 1996; Til and van Nesselrooij, 1994).

Dogs: In a 1-year dietary study, four male and four female beagle dogs were administered erythritol in the diet at concentrations of 0 (control), 2, 5 or 10% (Dean and Jackson, 1992; Dean *et al*., 1996). Animals were observed daily, and measurements were made of body weights, food and water consumption, haematology, blood chemistry, faecal analysis and urinalysis parameters, including urinary enzymes. Ophthalmological examinations were performed pre-dosing and during weeks 13, 26, 38 and 52. At necropsy, all animals were subject to gross pathological examinations, key organs were weighed, and all major tissues processed histopathologically.

No effects attributable to erythritol treatment were found relating to mortality, clinical signs, body weight gains, food intake, or on the results of ophthalmological examinations, haematological or blood chemistry parameters. Sporadically, statistically significant changes in haematological and blood chemistry parameters were reported; however, these observations were considered to be unrelated to treatment since they were slight, inconsistent across time, dose and sex, and were within the normal range of values for beagle dogs. Moreover, certain of the clinical chemistry values for the controls lay at the extremes of these ranges.

Treatment of beagle dogs with 2, 5 or 10% erythritol mixed in the diet appeared to be associated with increased water intake and increased urine volumes at the high-dose level, although there were large inter-individual variations. During week 13, decreased 24-hour urinary excretion of sodium and calcium was reported for the mid- and high-dose males. Reduced excretion of magnesium in high-dose males and of magnesium and potassium in mid- and high-dose females also was recorded. At week 26, urinary excretion of lactate dehydrogenase in treated males and high-dose females and of GGT in low-dose animals and in high-dose males was increased. After correction for mmol creatinine, however, no significant effects on urinary parameters were apparent. Given this observation, the lack of any corroborating effects of treatment on clinical chemistry or histopathological parameters, and the large inter-individual variations which complicated statistical analyses, the reported changes in the non-creatinine normalized urinary parameters were considered to be of no toxicological significance.

As seen in many of the rodent studies, slightly higher absolute and body weight adjusted kidney weights were recorded in the high-dose animals. Organ weight analyses also revealed increased absolute prostate weights in high-dose males associated with a slight increase in the incidence of cystic acini in the prostate of these animals. These findings were considered to be of no toxicological significance by the study authors since they may relate to individual variations in sexual maturity. Other statistically significant changes in organ weight parameters were reported, but were considered to be chance findings unrelated to erythritol treatment.

Overall, beagle dogs showed no overt signs of toxicity or ill-effects due to ingestion of erythritol at dietary concentrations of up to 10%, the highest dietary concentration tested. Moreover, there were no histopathological changes attributable to administration of erythritol.

Rats: In a 78-week study (Til and van Nesselrooij, 1994), erythritol of greater than 98.5% purity was administered in the diet to groups of 20 Wistar (Crl:WI(WU)BR) rats at concentrations of 0 (control), 1, 3 or 10%. A group of 10 reserve animals of each sex was included in the study design. Throughout the study the animals were observed for signs of ill-health and subject to weekly palpation, ophthalmological and haematological examinations, clinical chemistry analyses, and urinalyses. Body weight gain and food and water consumption were recorded regularly. At necropsy, organ weight determinations were made for the adrenals, brain, caecum (full and empty), heart, kidneys, liver, ovaries, pituitary, spleen, testes and thyroid. At this time, all animals were subject to complete gross and histopathological examinations.

As a result of treatment, soft faeces were observed in the high-dose animals during the first weeks of the study, but resolved thereafter. Behaviour and appearance of the rats were unremarkable for the first year, with age-related changes noted in all groups, including controls, in the remaining 6 months of the study. During the course of the study, two control males, six high-dose males, one control female and three low-dose females died or were killed *in extremis*. The deaths were not considered treatment related. The deaths were generally the result of various infections or were due to subcutaneous masses.

Body weights were statistically reduced compared with controls in the high-dose males, with lesser reductions in body weight gains in the high-dose females. Food intake was significantly increased at several time points in both sexes of the 3% dose group. Lesser increases were reported for the high-dose groups. No significant differences in food conversion efficiency were recorded. Water intake increased in a dose-dependent fashion, generally attaining statistical significance at most time points in the high-dose groups, but only at several time points in the lower dose groups. The reduced body weight gain in the highest dose groups despite a

slightly higher food intake was attributed to the lower caloric content of erythritol and not the result of any specific toxic effect.

A number of isolated, statistically significant, changes in haematological parameters occurred; however, they were considered to be isolated chance findings and therefore unrelated to erythritol treatment. Similarly, although a number of significant changes in blood chemistry parameters were recorded during the study, only the increases in alkaline phosphatase activity in the high-dose animals were considered to be related to treatment. Reported increases in serum GGT activity in the mid- and high-dose females at week 13 were considered to be due to the abnormally low activity in the controls. The remaining changes in clinical chemistry parameters either showed no dose–response, lacked consistency across sex and time or were within the normal range of variation, and as such were not considered to be toxicologically significant.

Slightly increased urinary output was attributed to the increased load on the kidneys brought about by the high renal clearance of erythritol. Increased water intake occurred in a dose-dependent fashion to compensate for this change. The slight nature of this effect in this study was evidenced by the lack of a dose-dependent significant increase in absolute or relative kidney weights, effects which were generally observed in the subchronic rat studies. Calcium excretion over a 16-hour period was increased in the 10% erythritol groups. This effect was thought to be possibly due to increased calcium absorption from the gut, a phenomenon reported with certain other low molecular weight organic compounds which are fermented in the colon. The increases in calcium excretion were considered to be of no toxicological significance since nephrocalcinosis was not observed, and no histopathological correlates were observed in the kidney.

Til and van Nesselrooij (1994) reported that erythritol treatment was associated with increased absolute and relative caecal weights at the 10% dose level and to a lesser extent at the 3% dose level. This effect was observed in most other rodent studies with erythritol and was attributed to an increased amount of osmotically active substances (i.e. erythritol and short-chain fatty acids resulting from microbial fermentation of erythritol which were not absorbed in the small intestine) in the large intestine. Increased ALP activity, an effect reported in short-term rat studies, was also ascribed to changes associated with caecal enlargement as the intestine is considered to be the main source of this enzyme in non-fasting rats (Righetti and Kaplan, 1971). Also, other substances which produce an increased osmotic load to the intestine have been demonstrated to produce a concomitant increase in ALP activity (Bär *et al.*, 1995; Moser *et al.*, 1980; Schaafsma and Visser, 1980; Woutersen, 1987). Given that there were no histopathological effects in the large intestine attributable to treatment, the increased caecal weights and the increase in ALP activity were considered to represent physiological responses of no toxicological significance (Til and van Nesselrooij, 1994). A statistical evaluation of the histopathological data revealed no potential adverse effects of erythritol treatment and no evidence of carcinogenic activity was found.

Essentially no significant toxicity was attributed to erythritol treatment although physiological responses, including decreased body weight gain, increased urinary volumes, caecal enlargement, and increased plasma ALP activity were observed. Even at the 10% dose level, the highest dose tested, erythritol was essentially non-toxic. Also, despite the longer treatment period in this study, the observed effects of erythritol were essentially similar in scope and severity as those reported in 28-day and 13-week toxicity studies and were of a type and nature that one would expect following oral administration of a low molecular weight, well-absorbed and rapidly excreted compound having significant osmotic activity.

The results of a 2-year rat study have further demonstrated that doses of erythritol up to 5.4 g/kg body weight/day for 2 years produce no evidence of toxicity or carcinogenicity (Lina *et al.*, 1994, 1996). In this study, groups of male and female Wistar rats were fed 0, 2, 5 or 10% erythritol in the diet (approximately 0, 0.86, 2.2 or 4.6 and 0, 1.0, 2.6 or 5.4 g/kg body weight/day for male and female rats, respectively) for 105 to 107 weeks. Another group was fed 10% mannitol in the diet.

There were no effects on the general condition or behaviour of treated animals. Food consumption was increased in high-dose animals and water consumption increased in the high-dose animals and in the mid-dose males. Rats administered 10% mannitol were also found to show evidence of increased water consumption. Compared with the controls, body weights were significantly reduced in the high-dose animals throughout the study. Reduced body weight gains were also reported for lower dose males and in rats treated with mannitol. There were no clinically relevant effects of erythritol treatment on haematological or blood chemistry parameters.

Urine volume was found to be significantly increased in the 10% erythritol group throughout the study, except at week 102. As would be expected with increased urine volume, urine osmolarity was decreased in the high-dose animals, although this occurred for the first year of the study only. These urinary changes were accompanied by a significant increase in excretion of NAG. There were also slight increases in urinary excretion of low molecular weight protein, total protein and electrolytes, including sodium, potassium, phosphate and calcium at the 10% erythritol dose level.

Increased urinary citrate, which normally accompanies higher calcium levels, was also present with both erythritol and mannitol. As a result of the increased excretion of calcium in the urine, nephrocalcinosis occurred, mostly in females. The degree of nephrocalcinosis seemed slightly greater in mannitol-fed rats since slight pelvic epithelial hyperplasia was also seen in this group. The finding of nephrocalcinosis is not unexpected in cases where there is increased excretion of calcium for long periods of time. As previously discussed, the increase in calcium excretion is possibly due to increased absorption, an effect which has been demonstrated to occur with certain poorly absorbed or poorly metabolized carbohydrates (Bär, 1985; Dills, 1989; Roe, 1989; Senti, 1986).

The urinary excretion of the renal brush-border enzyme GGT was significantly increased in the first year of the study (week 50) in the 5 and 10% erythritol groups. Increased GGT continued to week 78 in the 10% group only, but was similar to controls at week 102 indicating that this effect was not progressive. Increased urinary excretion of GGT was probably the result of diuresis, a known cause of increased urinary excretion of this enzyme (Burchardt and Jung, 1992). As previously discussed for a 13-week rat study (Til *et al.*, 1996), increases in urinary GGT excretion have been documented to occur with administration of substances which result in increased urine flow.

As also reported in previous rodent studies, caecal weights (full and empty) were significantly increased in the high-dose rats and occasionally in the mid-dose rats. Although relative kidney weights also tended to be increased in rats in the 10% erythritol group in the 2-year study (relative kidney weights were significantly increased in males at weeks 52 and 78, but not week 104, and in females at week 104), histopathological examination of all organs was unremarkable except for a statistically significant increase in the incidence of pelvic nephrocalcinosis in females of all erythritol groups and in both male and female rats in the 10% mannitol group (Lina *et al.*, 1996).

Reproductive toxicity studies. To further evaluate the safety of use of erythritol in foods, the potential for erythritol to have adverse effects on reproductive performance has been examined in two mouse studies, one utilizing the oral route of exposure (Tateishi *et al.*, 1989) and the other the intravenous route (Tateishi *et al.*, 1992), as well as in one two-generation rat study (Smits-van Prooije *et al.*, 1996a; Waalkens-Berendsen *et al.*, 1996).

Mice: In the first study (Tateishi *et al.*, 1989), erythritol was administered by gavage to groups of 24 male and 24 female Crj:CD-1 (ICR) strain mice at doses of 0 (control—distilled water), 1, 2, 4 or 8 g/kg body weight/day. Males were treated daily for 9 weeks then mated with females and continued to be dosed until vaginal plugs were observed. Females were dosed daily for a minimum of 15 days prior to mating and until day 6 of gestation and then observed until day 18 of gestation and killed prior to giving birth, or, if giving birth prior to day 18, killed at the time of birth. Mice were observed daily for clinical signs. Body weights and food and water consumption rates were recorded throughout the study. Pups from the terminated pregnancies were examined for the number of dead and live foetuses, with dead foetuses classified as either early (implantation scars and resorbed embryos) or late (macerated foetuses and dead foetuses at term). Live foetuses were weighed, sexed, and examined for external abnormalities. One-half of the live foetuses were preserved whole and the other half necropsied, with examination of the intraperitoneal organs and tissues. Paternal and maternal animals that were killed were subjected to gross pathological examination. The vagina and placenta of dams killed at day 18 of gestation were macroscopically observed and the *corpora lutea* and implantation sites counted. Selected parental animals also were subject to histopathological examinations.

Tateishi *et al.* (1989) reported that erythritol administration at 4 and 8 g/kg body weight/day was associated with an increased incidence of loose stools early in the dosing period. This effect largely resolved as dosing progressed. Increased water consumption was noted in most dose groups, probably stemming from the high degree of absorption of erythritol from the small intestine, its lack of metabolism, and near quantitative excretion in the urine. The diuretic action associated with this process may also have contributed to the finding of renal tubule dilatation in one of the high-dose males mated to a non-pregnant female.

There were no significant effects of erythritol treatment on reproductive performance. Implantation rate in the 4 g/kg body weight/day dose group and the male:female ratio in the 8 g/kg body weight/day dose group were significantly lower than in the controls; however, there was no clear dose–response pattern and both parameters were within the normal historical range. No effects on the foetuses were observed. There were no treatment-related histopathological effects in the maternal or paternal animals, other than possibly the dilatation of renal tubules in one high-dose male. Tateishi *et al.* (1989) concluded that erythritol treatment to 8 g/kg body weight/day, the highest dose tested in this study, produced no adverse effects on reproduction or on foetal development.

A second study of similar design to Tateishi *et al.* (1989) also was performed, but used the intravenous route of administration (Tateishi *et al.*, 1992). The doses administered were 0 (physiological saline), 1.0, 1.73 or 3 g/kg body weight/day.

The administration of up to 1.73 g/kg body weight/day was without effect, other than physiological responses noted at the injection site. At a

dose of 3 g/kg body weight/day, one male and one female died, probably as a result of osmotic overload leading to circulatory collapse. This effect of highly osmotic solutions dosed intravenously has been previously documented in an acute study (Yamamoto *et al.*, 1987). In the high-dose males, reduced motility and decreased body temperature also were observed. Water intakes were found to be increased, but not significantly, in high-dose females, and to a lesser extent in high-dose males. Pathological examination of the parental animals revealed slight dilatation of the renal tubules in one high-dose male and two high-dose females. Dilatation of the Bowman's capsule was also reported in one high-dose male. The high osmolality of the dosing solution was likely to be responsible for these observations through the induction of diuresis as evidenced by the increase in water consumption reported in the high-dose animals.

At the high doses tested, there were no effects of treatment on reproductive performance, including copulation, pregnancy and gestation rates. Also, there were no adverse effects of erythritol treatment on indices of foetotoxicity and teratogenicity.

Rats: To further examine the safety of erythritol, a two-generation reproduction rat study was conducted (Smits-van Prooije *et al.*, 1996a; Waalkens-Berendsen *et al.*, 1996). Groups of 24 male and 24 female rats were fed erythritol at dietary concentrations of 0, 2.5, 5 or 10% (approximately 0, 1.4 to 3.8, 2.8 to 7.5 or 6.5 to 16 g/kg body weight/day, respectively, depending on the stage of the pregnancy) for approximately 10 weeks prior to mating and during gestation. Twenty-four offspring of each sex and dose group formed the second generation and were treated in a manner similar to the parental generation. For each generation, one litter was reared to 21 days.

As a result of treatment, loose stools were observed in the high-dose F_0 animals during the early part of the study. No signs of laxation were seen in the F_1 generation. Compared with the controls, body weights generally were reduced in the 10% dose groups of both generations. Slight body weight decreases were seen in the high-dose females; however, there were no differences during gestation and lactation. Food intake was increased in the erythritol-treated animals. The reported decreased body weight gains despite generally higher food intake were ascribed to the reduced caloric value of the diet. Erythritol did not affect the reproductive performance or fertility of the parental rats. Also, there were no effects on the development of the offspring. As had been previously reported in both the unpublished and published feeding studies on erythritol, histopathological examination revealed no abnormalities.

Teratology studies. In addition to the reproductive toxicity studies, several experimental animal studies have been performed specifically to address the embryotoxic and/or teratogenic potential of erythritol at high doses. One study was performed in rats using the oral route of exposure (Smits-van Prooije, 1993; Smits-van Prooije *et al.*, 1996b), while two others were performed using intravenous injection, one in mice (Ota *et al.*, 1990) and one in rabbits (Hashima Laboratory, 1989; Shimizu *et al.*, 1996). These studies, described below, demonstrate that erythritol is non-foetotoxic and non-teratogenic.

Rats: In the study reported by Smits-van Prooije *et al.* (1996b), groups of 32 pregnant Wistar rats were fed 0, 2.5, 5 or 10% erythritol in the diet (approximately 0, 1.7, 3.3 or 6.6 g erythritol/kg body weight/day) during gestation days 0 to 21. No mortality occurred, and weight gain during gestation, food consumption, food efficiency and reproductive performance were similar in all groups, with the exception of reduced weight gain in the animals of the high-dose group during the second week of gestation. No clinical signs, including evidence of laxation, were noted in the treated parental animals.

In terms of reproductive parameters, foetal development, and litter parameters, no apparent treatment-related changes were observed in the study. Among the statistical differences observed, none was indicative of toxicity, developmental or otherwise. An observation of higher post-implantation loss in the low-dose group was not accompanied by similar differences in the higher dose groups and, therefore, was considered not to have been treatment related. The data concerning ossification produced some statistically significant differences, but overall, the degree of ossification was comparable among the high-dose animals and controls.

Smits-van Prooije *et al.* (1996b) concluded that no adverse reproductive, embryotoxic, foetotoxic or teratogenic effects occurred as a result of administration of erythritol in the diet at levels of up to 10% (equivalent to approximately 6.6 g/kg body weight/day) and that only slight maternal effects (lesser weight gains due to the reduced caloric value of the diet) occurred at this dose level. No effects were associated with administration of erythritol at the lower dose levels (i.e. at or below 3.3 g/kg body weight/day).

Mice: The lack of embryotoxic and teratogenic activity of erythritol also was demonstrated in a mouse study (Ota *et al.*, 1990). In this study, erythritol was administered by intravenous injection to groups of 42 female pregnant SPF Crj:CD-1 (ICR) mice at doses of 0 (control), 1, 2 or 4 g/kg body weight/day on days 6 through 15 of gestation. The group of 42 males mated to the females were untreated. Following dosing, 27 mice per dose group were used for evaluation of pups at termination of pregnancy on day 18 of gestation, 15 mice per dose group were allowed to deliver naturally, with their pups used for developmental and reproductive evaluations. Some of these pups were mated

at 10 weeks post-partum and their offspring subject to similar evaluations as performed on the F_1 foetuses.

Erythritol administration was associated with transient clinical signs, including hypoactivity and staggering gait, directly after intravenous dosing at 4 g/kg body weight/day. Two deaths in the high-dose group during or directly after dosing were considered treatment related; however, since specific toxic effects were not observed at necropsy, the deaths of these animals were considered to be due to an osmotic imbalance created by erythritol since the intravenous dosing solution was approximately equivalent to one-quarter to one-third of the animal's total blood volume. Similarly, the observation of increased water intake during the latter period of dosing was considered to be the result of the known diuretic action of erythritol.

In terms of reproductive performance, no significant changes considered to be treatment related were observed. Premature births occurred only in the treated groups (five low-dose, one mid-dose, one high-dose); however, neither the incidence nor the dose–response aspects suggested a treatment-related effect. The total incidence of external abnormalities was slightly increased ($P > 0.05$) in the pups from the high-dose dams. Also in this group, there was a statistically significant increase in the total incidence of skeletal variations, such as wavy ribs and fusion of sternebrae. Based on these findings, Ota *et al.* (1990) concluded that there was a slight effect of erythritol on foetal development; however, they reported that the incidences of specific external or skeletal abnormalities were not increased relative to controls. Also, there were no increases in the incidence of 14th ribs, which is considered to be a relatively sensitive endpoint for assessing teratogenic effects (Khera, 1981). Furthermore, Ota *et al.* (1990) also noted that no significant effects on external or skeletal abnormalities were seen when erythritol was administered by intravenous injection to rabbits (Hashima Laboratory, 1989), a species considered to be more sensitive than mice to teratogens (Ota *et al.*, 1990). Consequently, although certain changes appeared to be erythritol related, they were considered to be of a non-specific nature and likely to be due to the hypertonicity of the concentrated dosing solution. No abnormalities in visceral organs were observed in this study (Ota *et al.*, 1990).

The development of pups, as assessed by numerous tests, was not affected by erythritol treatment nor were there any indications of impaired reproductive performance in F_1 animals or of any changes in their offspring. Ota *et al.* (1990) concluded that the effects seen in the high-dose group during treatment were due to the osmotic properties of the test substance. Slight indications of a general effect on developmental status were thought to be related to the hypertonicity of the test substance.

Rabbits: As cited by Ota *et al.* (1990), erythritol administered by intravenous injection to rabbits has shown no indication of embryotoxic or teratogenic potential (Hashima Laboratory, 1989). The results of this unpublished study were described in a recently published report (Shimizu *et al.*, 1996). In this study, groups of 17 female KBL:JW strain rabbits were injected intravenously with 1.0, 2.2 or 5.0 g erythritol/kg body weight/day during days 6 to 18 of gestation. Males to which females were mated were not treated. Pregnancies were terminated on day 28 of gestation. Observations of the dams included daily examination for clinical signs of ill-health and measurement of body weight gains and food and water consumption rates. All animals surviving until day 28 of gestation were killed and grossly examined. Selected organs were removed and weighed. Dams that were not pregnant were killed and discarded, but, as with pregnant females, any abnormal tissues were preserved. All animals dying during the study were necropsied to confirm pregnancy. Also, in those with confirmed pregnancy, the ovaries and uterus were removed and the number of implantations and *corpora lutea* were counted. For each dam, the numbers of dead, resorbed foetuses and live foetuses were counted. Dead or resorbed foetuses were classified and discarded. Live foetuses were examined for external abnormalities, weighed and sexed. Approximately one-half of the live foetuses were examined for skeletal changes and one-half examined for abnormalities of the visceral organs.

Although transient maternal effects (i.e. polyuria, auricular oedema, bradypragia and emaciated appearance), probably related to increased osmotic load, were observed at the highest dose tested, no effects on foetal development as a result of treatment were found. There were no differences in body weight or food consumption in treated maternal animals compared with controls. Water consumption was increased in a dose-related manner in all treated groups. The lack of any effects on dams after the dosing period provides further evidence to suggest that these findings were related to the physiological effects of erythritol, namely osmotic loading and diuresis, and do not represent true toxicological sequelae. This conclusion is further supported by the lack of finding of treatment-related histopathological effects. Skeletal and visceral examinations of the foetuses revealed no treatment-related teratogenic effects.

Mutagenicity studies. Erythritol does not contain any functional groups which would provide any suspicion of mutagenicity. Erythritol has a high degree of water solubility, is not metabolized, and is rapidly excreted following absorption, a spectrum of properties not generally associated with mutagenic activity. The expected lack of mutagenic activity of erythritol is also supported by the results of 78-week and 2-year rat studies (Lina *et al.*, 1996;

Til and van Nesselrooij, 1994) which provide no evidence of carcinogenicity. Moreover, a number of related substances have been reported to show no mutagenic activity either *in vitro* or *in vivo* (Lynch *et al.*, 1996).

To confirm the anticipated lack of mutagenic potential, erythritol has been subject to two Ames' tests (Blijleven, 1990; Kawamura *et al.*, 1996) and an *in vitro* chromosome aberration test in Chinese hamster fibroblasts (Kawamura *et al.*, 1996).

In the first Ames test (Blijleven, 1990), erythritol at concentrations of 0.37–30 mg/plate showed no mutagenic activity when tested in five histidine-requiring *Salmonella typhimurium* strains (TA1535, TA1537, TA1538, TA98 and TA100) using the Ames assay, with or without metabolic activation by a liver S-9 fraction from Aroclor-induced rats. In addition, erythritol showed no evidence of toxicity to *S. typhimurium* at the maximum dose tested of 30 mg/plate, both in the absence and in the presence of the exogenous mammalian metabolic activation system. Positive controls were reported to induce the expected increases in reverse mutant colony frequency.

The results of the second Ames assay also showed erythritol to be non-mutagenic following incubation with histidine-requiring *S. typhimurium* strains TA98, TA100 and TA1537 or with *Escherichia coli* strain WP2 *uvrA* at doses of up to 5000 μg/plate, both in the absence and presence of an exogenous source of metabolic activation (Kawamura *et al.*, 1996). In strain TA1535, in the presence of S-9, several of the dose levels exhibited greater numbers of revertant colonies than the controls. These increases, however, failed to a show a dose–response relationship and showed considerable variation in number. As a result, these increases were not considered to provide evidence of mutagenic activity. There was no evidence of cell toxicity, even at the maximum concentration tested.

In an *in vitro* chromosome aberration assay in Chinese hamster fibroblast cells, erythritol at concentrations of up to 10 mmol did not produce a significant increase in the incidence of abnormal cells, polyploid cells, total chromosomal aberrations or in any individual type of chromosomal aberration (Kawamura *et al.*, 1996; Nakatsuru *et al.*, 1988). In addition, erythritol was found to be non-toxic to the cultures up to the maximum concentration tested of 10 mmol.

On the basis of the results of the available *in vitro* Ames and *in vitro* chromosome aberration assays, erythritol is concluded to be devoid of mutagenic activity.

Clinical studies

The clinical program for erythritol involved a series of single-dose and repeat-dose, short-duration studies which have been used to investigate the human correlates to the physiological responses seen in the preclinical studies. In total, 11 clinical studies on erythritol were performed. In all cases, the clinical studies show erythritol to be well tolerated and not to cause any toxicologically relevant effects, even following high-dose exposure. The clinical studies are briefly described in Table 5 which, to avoid confusion, includes both the unpublished and published referencing notations.

As previously discussed, the metabolism of erythritol in healthy subjects was investigated in several clinical studies (Bornet *et al.*, 1996a,b; Hiele *et al.*, 1993; Ishikawa *et al.*, 1992, 1996; Noda *et al.*, 1988, 1994). Briefly, erythritol administered orally to humans was rapidly absorbed from the gastrointestinal tract and quantitatively excreted in the urine without undergoing metabolic change. At high oral doses, urinary excretion accounted for approximately 90% of the administered dose with minimal amounts appearing in the faeces, indicating some degree of utilization by intestinal bacteria. A comparison of the human and animal data demonstrated a high degree of similarity in the essential metabolic characteristics of erythritol and supports the use of the animal species used to evaluate its safety for human consumption.

Gastrointestinal tolerance

The data from the preclinical studies indicated that there was a potential for large doses of erythritol to cause some alterations in water balance which might result in minor gastrointestinal effects, namely laxation, and increased water intake, urinary output, and excretion of electrolytes in humans. As a result, investigation of such effects was a main focus of several of the clinical studies. Investigation of possible laxative effects and gastrointestinal tolerance was performed in most of the clinical studies, with four studies focusing on this issue (Oku and Okazaki, 1996a,b; Takahashi, 1992a,b; Umeki, 1992), and two others incorporating these endpoints into more definitive clinical investigations (Bornet *et al.*, 1996b, Tetzloff *et al.*, 1996).

Two of the studies focusing on laxation were conducted using groups of healthy volunteers who consumed erythritol in solution at bolus doses progressing from 30 to 60 g/day, with wash-out intervals between administration of successive dose levels (Takahashi, 1992b; Umeki, 1992). Also ingested in each of these studies were fixed doses of sorbitol (10 or 30 g/day) and sucrose (10 or 60 g/day), which served as comparative controls.

In the third study, Oku and Okazaki (1996a,b) gave erythritol incorporated into jelly or dissolved in water to 38 volunteers at bolus doses which increased progressively from 25 g/day to 37.5, 50, 62.5 and 75 g/day, with progression from the lower doses occurring after a suitable wash-out period and only in the absence of loose stools at the lower

doses. Sorbitol, administered progressively starting at doses of 12.5 g/day, and sucrose, administered to each individual at the highest non-laxative dose of erythritol per subject, were used for comparative purposes.

In each of the above three studies, erythritol was administered once daily as a bolus dose in aqueous solution or jelly and generally over a short period of time (10 minutes) and on an empty stomach. Even under these exposure conditions, which are not representative of anticipated use patterns, only one or two subjects reported laxation at doses ranging from 0.62 to 0.7 g erythritol/kg body weight (Oku and Okazaki, 1996a,b; Takahashi, 1992b; Umeki, 1992). As a result, the reported findings of these studies are not indicative of potential for laxation under the expected conditions of use. Compared with sorbitol, the subjects' tolerance to erythritol was considerably greater, generally by a factor of three- to fourfold, since sorbitol produced laxation at doses in the range of 0.2 g/kg body weight.

Under the expected conditions of use, erythritol would be consumed as a component of food products at various times throughout the day. Under these conditions, the minimum effective doses required to produce laxation are expected to be greater than those reported by Umeki (1992), Takahashi (1992b) and Oku and Okazaki (1996a,b). This expectation is supported by the results of a fourth, 5-day repeat-dose study (Takahashi, 1992a) in which 40 g erythritol were administered in aqueous solution to eight healthy males and two healthy females. In contrast to the methodology employed in the other three studies (Oku and Okazaki, 1996a,b; Takahashi, 1992b; Umeki, 1992), the dose was divided into two 20-g portions which were administered 2–3 hours after breakfast and lunch, respectively. The total doses administered averaged approximately 0.64 and 0.74 g/kg body weight/day in males and females, respectively. No significant laxative effects were reported at these average dosing levels.

In three additional clinical studies (Bornet *et al.*, 1996a,b; Tetzloff *et al.*, 1996), gastrointestinal tolerance and laxation were evaluated as part of a larger study. In the first study (Bornet *et al.*, 1996a), six subjects who fasted overnight ingested a single bolus dose of a 250-ml solution containing 1 g erythritol/kg body weight. Two of these subjects reported loose stools and two others reported cramping, discomfort and flatulence. The occurrence of these effects was attributed, in part, to the method of administration since similar effects have not been observed when comparable doses were administered in food over the course of a day (Tetzloff *et al.*, 1996; see below).

In a second study (Bornet *et al.*, 1996b) using six subjects per group, single doses of 0.4 or 0.8 g erythritol/kg body weight, incorporated into chocolate, were ingested approximately 2 hours after a fixed breakfast. Control groups received chocolate containing sucrose at a dose of 0.8 g/kg body weight or did not receive any chocolate snacks. At the higher dose level of 0.8 g erythritol/kg body weight, gastrointestinal effects, consisting of flatulence, rumblings and nausea, were reported; however, the incidence of these effects was not increased relative to either the negative or sucrose controls. No changes in water intake or faecal characteristics were observed at either dose level of erythritol.

In a third clinical study using 12 healthy male subjects in a double-blind cross-over manner, erythritol or sucrose was incorporated into foods at levels providing up to 1 g/kg body weight/day for a treatment period of 7 days (Tetzloff *et al.*, 1996). Gastrointestinal tolerance towards erythritol and sucrose was unremarkable, with a few subjects noting flatulence, bloating and a sensation of fullness with each article. The incidence of gastrointestinal effects was higher with sucrose compared with erythritol. Liquid intake was higher on average (2.60 litres/day) during the entire study (i.e. both during erythritol and sucrose periods) compared with the reported normal liquid intake (1.80 litres/day) reportedly due to the hot weather at the time of the study, but no significant differences between the sucrose and erythritol treatments were observed. Water intake was similar (2.60 litres/day) during sucrose treatment and erythritol treatment (2.58 litres/day). The subjects' perception of stool and urinary excretion indicated that stool was slightly softer, but slightly less in quantity with erythritol than during sucrose treatment; however, no statistically significant effects were found. Frequency of defaecation, and urine quantity and frequency during erythritol ingestion were not perceived to be different from sucrose ingestion.

When the results of all clinical studies are considered, it is apparent that repeated ingestion of erythritol with food tended not to show transient gastrointestinal effects when compared with acute ingestion of erythritol in solution on an empty stomach. Moreover, any transient gastrointestinal effects that were reported were observed only when erythritol was administered in a bolus liquid form on an empty stomach in a short period of time (Bornet *et al.*, 1996a). At a dose of 1 g/kg body weight/day, no significant gastrointestinal effects were observed when erythritol was ingested with food and beverages over the course of a day (Tetzloff *et al.*, 1996). The finding of a relatively higher incidence of gastrointestinal effects with acute bolus dosing in solution and/or on an empty stomach probably reflects greater passage of the osmotically active erythritol into the large intestine as a result of reduced absorption.

Effects on biochemical and urinary parameters

In addition to the evaluation of gastrointestinal tolerance, clinical studies were also conducted to evaluate the overall safety of erythritol under the expected conditions of use. Based on the preclinical studies, any effects expected from erythritol ingestion would likely be a physiological response to alterations in water balance.

Three studies were performed specifically to investigate the potential influence that the ingestion of erythritol might have on carbohydrate metabolism in non-diabetic individuals (Noda *et al.*, 1994) and in diabetics (Ishikawa *et al.*, 1992, 1996; Miyashita *et al.*, 1993). In non-diabetic individuals, erythritol was dissolved in water and ingested at a single dose of 0.3 g/kg body weight (Noda *et al.*, 1994). One week later, glucose was ingested at the same dose level. Analysis of blood performed at 0.5, 1, 2, 3, 8 and 24 hours after erythritol administration showed no changes in insulin or glucose concentrations whereas both parameters were increased within half an hour of glucose ingestion, returning to basal values within 3 hours. Erythritol was not associated with any changes in serum cholesterol, triacylgylcerol, free fatty acids, sodium, potassium or chloride levels. Urinary volume, osmotic pressure, and concentrations of sodium, potassium and chloride were not significantly different after ingestion of erythritol. Excretion of erythritol in the urine accounted for approximately 90% of the administered dose within 48 hours of dosing.

In diabetics, erythritol, in a single oral dose of 20 g dissolved in 100 ml of water, was ingested by five fasted patients followed by the ingestion of normal meals approximately 3 and 12 hours later (Ishikawa *et al.*, 1992). Serum insulin, glucose, non-esterified fatty acids (NEFA), 3-hydroxy butyric acid (HBA), and erythritol concentrations were measured prior to and for up to 24 hours after erythritol administration with all parameters analysed in comparison to pre-dosing values. Urinary erythritol output was measured during each of the three consecutive 24-hour periods immediately after dosing. Analysis of the serum values showed that neither insulin nor glucose concentrations changed in response to erythritol administration whereas both were significantly increased, as would occur normally, following ingestion of food. The other indicators of carbohydrate metabolism (NEFA, HBA), both gradually increased after dosing, with NEFA becoming statistically increased just prior to ingestion of the first meal. After consuming the meal, both NEFA and HBA decreased to levels that were significantly ($P < 0.05$) lower than pre-dosing levels. These changes, which initially indicated a shift towards utilization of fat stores followed by a reversal towards carbohydrate utilization after the meal, were consistent with the fasting state of the subjects at the time of dosing and were not a consequence of the administration of erythritol.

In another study involving diabetics, 11 patients (three male and eight female) consumed 20 g erythritol/day for 14 days (Miyashita *et al.*, 1993). Erythritol was added to foods or drinks, with the 20 g dose consumed each day without any specific division or timing. Prior to and at the end of the dosing period, body weights and serum blood sugar (fasting), haemoglobin A1c (i.e. glycosylated haemoglobin), blood urea nitrogen, creatinine, and β_2-microglobulin were measured, as were urinary proteins. All patients kept records of any physical effects or changes which occurred over the test period and of all foods consumed for the first 3 and last 3 days of treatment. Analysis of the body weight and renal function data (i.e. both plasma and urine indicators) did not reveal any changes relative to baseline values nor was laxation or any subjective symptom reported. BUN, serum creatinine, β_2-microglobulin and urinary proteins were not affected by treatment. Both fasting blood sugar and serum haemoglobin A1c were decreased at the end of the study. These changes were seen to occur to the greatest degree in patients with high pre-dosing levels and, since decreases in both parameters would indicate an advantageous effect in diabetics, they indicate a potential benefit to the use of erythritol.

The studies in diabetics demonstrate that erythritol does not adversely affect carbohydrate metabolism. Further, the urinary elimination and renal function data in these studies show that the kinetics of and reaction to erythritol are, for all practical purposes, identical to those of non-diabetic individuals. Together, these facts suggest that erythritol could be used in foods consumed by diabetics.

Bornet *et al.* (1996b) fed a single dose of 0.4 or 0.8 g erythritol/kg body weight to human subjects as a chocolate snack. A control group received chocolate providing 0.8 g sucrose/kg body weight. Serum glucose, insulin, sodium, potassium, chloride, creatinine, osmolarity, haematocrit and albumin were measured hourly from 2 hours prior to dosing until 8 hours after dosing, and urinary volume, creatinine, sodium, potassium, chloride, osmolarity, urea, NAG and GGT were measured every 2 hours, starting from 2 hours prior to dosing until 8 hours after dosing. No changes in any blood parameters were observed with erythritol, whereas sucrose caused increases in both blood glucose and insulin concentrations directly after ingestion, as would be expected. For the urinary parameters, osmolarity and excretion of chloride and sodium were increased in both the low- and high-dose erythritol groups, although for chloride, the difference relative to sucrose was not statistically significant and, for the other variables, was not as great when compared with the sucrose group as to the negative

control. Although the urinary changes were not marked, they followed both a dose- and time-dependent course (i.e. lasting up to 4 hours in the low-dose group and up to 6 hours in the high-dose group), indicating an effect due to erythritol that was greater than that elicited by sucrose. Urinary volume also was slightly increased in the high-dose erythritol group but a greater increase was seen with sucrose. Urinary levels of NAG were not altered by either erythritol or sucrose administration. A critical evaluation of the GGT data indicated that the reported findings were uninterpretable (Bernt *et al*., 1996). In the first instance, subsequent studies (Loeb *et al*., 1997) have demonstrated that under the conditions that the urine samples were stored GGT is unstable. Moreover, erythritol has been shown to enhance the stability of GGT under these same storage conditions (Loeb *et al*., 1997), making any comparisons with control values impossible. In any event, the GGT data would add little to the assessment of erythritol since urinary NAG activity is considered to be a superior indicator of renal integrity (Price, 1982, 1992).

Oku and Okazaki (1996a,b) administered up to 75 g erythritol/day in jelly or liquid to 38 volunteers. No significant changes of blood levels of total and specific blood and liver function proteins, cholesterol, triglycerides, urea and uric acid, electrolytes, glucose, and red and white cell parameters were reported.

A repeat-dose clinical study of the potential for erythritol to cause minor changes in renal parameters was recently reported by Tetzloff *et al*. (1996). In this study erythritol, incorporated into foods, was consumed in a manner similar to that expected to occur during normal use, was evaluated in a 14-day, double-blind crossover study using 12 healthy male subjects. The study consisted of two consecutive 7-day periods in which the subjects randomly assigned to two groups, consumed either erythritol or sucrose for the first 7 days followed by the other product for the second 7 days. The first 2 days of each period were used as adaptation periods during which the subjects ingested, without supervision, 0.3 and 0.6 g of substance per kg body weight on day 1 and day 2, respectively. During the remaining 5 days of each 7-day period, the subjects were institutionalized, and ingested, under supervision, full doses of 1 g/kg body weight/day. The daily doses were consumed in and by the following foods and schedule: 20% in yogurt and biscuits during breakfast, 10% in biscuits during morning coffee, 30% in soft drinks during lunch, 20% in chocolate during the afternoon, and 20% in yogurt during dinner.

Each subject in the study was screened for specific diseases, allergies, drug/alcohol and medication use, or other conditions which might affect the outcome of the study. During the adaptation period of ingestion at the beginning of each 7-day exposure period, daily food consumption records were kept by each subject, while during the supervised intake periods, all subjects consumed identical meals. During the full-dose periods, sensory perception of foods, overall well-being, feelings of hunger and thirst, desire for sweet or salty foods, perception of regularity, consistency and quantity of stool, frequency and quantity of urine, gastrointestinal symptoms, side-effects, fluid and food intake, body weight and blood pressure were recorded. Urine samples were taken at 7.30 am on the days before and at the end of the 7-day test periods and on the last 4 days of the supervised periods. The latter samples were collected over 3-hour periods from 10 am to 10 pm and for the overnight period from 10 pm to 7 am the next day. Urine volume, pH, conductivity, osmolality, low-molecular weight protein, GGT, NAG, β-microglobulin, albumin, creatinine, sodium, potassium, chloride, calcium, phosphate, citrate, urea and erythritol concentrations were measured for each urine sample, and excretion was calculated both on an absolute basis and relative to creatinine excretion.

Over the course of the study, no changes were seen in body weight or blood pressure, and feelings of hunger or thirst and desire for sweet or salty foods were unaffected by treatment. Analysis of sensory perception of food data showed that erythritol in cake did not alter its acceptance, while chocolate and yogurt were not as well accepted as with sucrose. Urinary volume was increased approximately 7% during erythritol treatment but was not statistically different from that during sucrose ingestion. Urinary osmolality and hourly output of osmotically active solutes were both statistically increased during the erythritol treatment period; however, the hourly increase (576 mOsm/24 hours) corresponded well with the expected increase calculated from the excretion of erythritol (62.4 g/24 hr = 511 mOsm/24 hours). Urinary conductivity was about 10% lower ($P < 0.005$) during erythritol treatment. Urinary phosphate output was slightly higher ($P < 0.1$ on an hourly basis; $P < 0.02$ relative to creatinine excretion) during erythritol treatment. Urinary calcium also was statistically higher, both on an absolute basis and relative to creatinine, during erythritol treatment; however, the difference was small, remained within the normal physiological range, and occurred during only one of the test weeks. Slight or statistically significant decreases were seen for urinary potassium, sodium and chloride concentrations, but the absolute excretion and rates relative to creatinine for these electrolytes were not altered.

Urinary protein and enzyme (NAG, β_2-microglobulin and albumin) excretion were statistically increased during erythritol treatment; however, numerically, the increases were small, were in the same range as during sucrose treatment and, impor-

tantly, remained within normal physiological values. As mentioned previously in respect to the Bornet *et al.* (1996b) study, the GGT data are considered to be uninterpretable.

Urinary excretion of erythritol, as determined during the sampling periods, ranged from 61 to 88% of the ingested dose for all participants and exceeded 70% in 11 of the 12 subjects. Analysis of diurnal variation showed that the highest excretion of erythritol occurred during the afternoon (4 to 7 g/hour) compared with the lowest excretion (0.5 to 2 g/hour) which occurred overnight. This reflects the rapid excretion of erythritol combined with the fact that administration occurred exclusively during the day or early evening.

In summary, a number of clinical studies have demonstrated that erythritol is well tolerated by both healthy and diabetic subjects and is without adverse effects. In these studies, urine output, water intake, and possible haematological and gastrointestinal side-effects were examined. The only effects reported in some subjects, but only at high doses, included small increases in urinary volume, electrolyte excretion, and water intake. These particular effects, however, are all related to the weak diuretic properties associated with high-dose exposure to erythritol and have been demonstrated in both the clinical and animal studies to be the result of physiological, rather than toxicological, responses. Moreover, those urinary parameters which were minimally affected by high-dose erythritol ingestion remained within the normal physiological range and, therefore, were not indicative of any pathological effects of erythritol treatment. Collectively, the clinical studies provide good evidence for the safety of use of erythritol in foods.

Discussion

Erythritol has been subject to extensive investigation in metabolic, toxicological and clinical studies as part of the evaluation of the safety of its use in foods. A number of metabolic studies and acute, subchronic and chronic toxicity studies in rats, mice and dogs, and several clinical studies in humans have been performed that demonstrate the safety of erythritol for use as a food ingredient. In all the studies, erythritol was well tolerated, even at very high doses, and was without adverse toxicological effects. Erythritol has been demonstrated to have no carcinogenic, mutagenic or teratogenic potential. Also, several studies have shown that at high doses erythritol has no effects on reproductive performance or fertility.

The metabolic data in both animals and humans show that erythritol is rapidly absorbed from the small intestine following oral ingestion and is excreted in unchanged form virtually quantitatively in the urine (Dean *et al.*, 1996; Lina *et al.*, 1996; Nakayama, 1990a,b; Noda and Oku, 1990; Noda *et al.*, 1996; Oku and Noda, 1990a,b; Til *et al.*, 1996; van Ommen and de Bie, 1990; van Ommen *et al.*, 1996). Erythritol does not undergo systemic metabolism. Based on urinary excretion data, the percent of ingested dose accounted for by absorption and renal excretion in humans and animals ranges from 60 to greater than 90% depending on the post-dose collection period, with rats and dogs reported to show a dose-dependent decrease in absorption at high oral doses (Dean *et al.*, 1996; Noda and Oku, 1992; Oku and Noda, 1990a). The unabsorbed fraction of ingested erythritol, particularly in rodents, is subject to microbial fermentation to volatile short-chain fatty acids in the large intestine (Noda and Oku, 1990, 1992). There are comparatively small fractions of ingested erythritol excreted in faeces or subject to biliary excretion.

Given the metabolic profile of erythritol (i.e. the rapid absorption and excretion as unchanged compound in the urine within 24 to 48 hours) and its low molecular weight, high water solubility, and its polar nature, high-dose exposures would be expected to produce physiological responses associated with osmotic diuresis. Similarly, the fermentation of unabsorbed erythritol in the colon would also be expected to result in physiological responses known to occur with other low molecular weight carbohydrates that reach the large intestine in significant quantities, either as a result of poor absorption or due to high-dose loading (Bär, 1985; Leegwater *et al.*, 1974; Newberne *et al.*, 1988; Smits-van Prooije *et al.*, 1990; Walker, 1978; WHO, 1987).

The spectrum of effects which would be predicted from the metabolic data have in fact been demonstrated to occur with erythritol in the acute, subchronic and chronic animal toxicity studies. These effects include decreased body weight gains as a result of the reduced caloric value of erythritol-containing diets, increased water consumption, urine volume, electrolyte excretion and increased absolute and/or relative kidney weights due to osmotic diuresis, transient occurrence of loose stools, increased absolute and/or relative caecal weights, and, in certain studies, increased serum alkaline phosphatase as a result of the loading of the large intestine with low molecular weight, osmotically active substances. In no study was histological evidence of toxicity reported. Moreover, in studies which allowed for a recovery period following the cessation of exposure, a reversal of the reported effects occurred (Kamata, 1990a,b; Yamaguchi *et al.*, 1990; Yamamoto *et al.*, 1989).

The effects reported in the toxicological and clinical studies were all considered to be physiological responses to osmotic loading and as such do not constitute toxicologically significant effects. In the analysis of toxicological data, a distinction must be made between toxic responses and reversible phys-

iological responses that are due to homeostasis-maintaining mechanisms (WHO, 1987). The World Health Organization (WHO) has indicated that laxative effects and increased caecal weights resulting from osmotic overload, and increases in renal weights due to processing of increased water volume under conditions of diuresis are examples of reversible changes that represent physiological responses rather than toxicological effects (WHO, 1987). These responses, in fact, are commonly observed with a number of poorly absorbed and/or poorly metabolized carbohydrate products and are regarded as not indicative of substance-related toxicity.

The increased absolute and/or kidney weights reported in many of the subchronic and chronic rodent studies, both by the oral (Lina *et al*., 1996; Shibata *et al*., 1991; Til *et al*., 1996; Yamamoto *et al*., 1989) and intravenous routes of exposure (Kamata, 1990a), as well as in one dietary dog study (Dean *et al*., 1996) were considered to be the result of the increased urine output found in these studies. Increased urine output would be expected to increase the workload on the kidney, increasing functional tissue activity and hence kidney weight. This is consistent with the WHO (1987) classification of such an effect as a physiological response. Diuresis, including diuresis resulting from high-dose exposure to carbohydrates (Bär *et al*., 1995), has been demonstrated in rodents to result in increased absolute and/or relative kidney weights (Ogino *et al*., 1994; Sterck *et al*., 1992).

That the increased kidney weights are a physiological response is further evidenced by the lack of significant histopathological findings in the kidneys other than slight dilatation of the renal tubules, an effect which would be expected for a substance with osmotic/diuretic activity (Tateishi *et al*., 1989, 1992; Yamaguchi *et al*., 1990; Yamamoto *et al*., 1989). Also, no increases in kidney weights or lesser increases were found at the end of studies which allowed for a recovery period after dosing was completed (Kamata, 1990a; Yamamoto *et al*., 1989). Similarly, in the rat and dog studies which included a recovery period, effects associated with the diuretic action of erythritol, including increased urine output and water consumption were found to be reversible upon cessation of the high-dose exposures (Kamata, 1990a,b; Yamaguchi *et al*., 1990; Yamamoto *et al*., 1989). As indicated by WHO (1987), the reversibility of an effect reported in a toxicological study is a hallmark of a physiological response.

Other effects which might be expected to be associated with a weak diuretic effect or with osmotic loading, including increased total excretion of electrolytes and urinary enzymes (e.g. GGT and NAG) (Mondorf *et al*., 1994; Obatomi and Plummer, 1993), were occasionally found in some studies (Dean *et al*., 1996; Lina *et al*., 1996; Til *et al*., 1992, 1996), although these findings were not consistent from study to study and were generally of a slight nature or failed to remain significant after normalization of the data to account for the rate of creatinine excretion. In no preclinical study were the effects on urinary parameters found to be progressive or to increase in severity over time. Based on these data, the increased kidney weights, minor changes in urinary parameters, and the increase in water consumption are concluded to be physiological responses to the diuretic action of high-dose erythritol exposure. As a result, no toxicological significance can be ascribed to these findings.

In the 2-year rat study (Lina *et al*., 1996), pelvic nephrocalcinosis occurred, particularly in females, due to the increased excretion of calcium. The degree of nephrocalcinosis appeared greater in a comparative study group administered mannitol. The finding of pelvic nephrocalcinosis in rodents is not unexpected when calcium excretion is increased for long periods of time and has been found to occur, generally of greater severity, with poorly absorbed or poorly metabolized carbohydrates (Bär, 1985; Conz and Maraschin, 1992; Dills, 1989; Roe, 1989; Roe and Bär, 1985; Senti, 1986). For these substances (Bär, 1985), and probably for erythritol, increased calcium excretion may partially be the result of increased calcium absorption due to the osmotic loading of the gastrointestinal tract.

In a few of the preclinical studies, increased BUN was reported in animals administered high doses of erythritol (Kamata, 1990a,b; Shibata *et al*., 1991; Yamamoto *et al*., 1989). Increased serum BUN may be associated with the loss of electrolytes, particularly sodium, resulting from the diuretic action of erythritol (Shibata *et al*., 1991). The association of increased serum BUN with electrolyte loss was effectively demonstrated in a 28-day study in which female rats were administered erythritol at high doses via oral gavage along with either tap water, low-electrolyte or high-electrolyte solutions. Rats administered the high-electrolyte solution along with erythritol showed no evidence of increased BUN while, in comparison to controls, those in the tap water and low electrolyte groups showed slight, although non-significant, increases in BUN. Based on these results, the reported increases in BUN were considered to be a physiological response to the diuretic properties of erythritol and not indicative of an adverse effect of erythritol treatment (Shibata *et al*., 1991).

Given the association between the reported slight increases in serum BUN (Kamata, 1990a,b; Shibata *et al*., 1991; Yamamoto *et al*., 1989) and the diuretic action of erythritol and noting: (a) the lack of evidence of pathological changes in the kidneys or other signs of impaired renal function; (b) the lack of finding of increased BUN in the chronic studies (Lina *et al*., 1996; Til and van Nesselrooij, 1994); and (c) the reversibility of increased serum BUN

following cessation of high-dose erythritol exposure (Kamata, 1990a,b; Yamamoto *et al.*, 1989), the slight increases in serum BUN reported in certain of the subchronic studies were considered to be of no toxicological significance.

The remaining spectrum of effects reported to occur in the preclinical studies, including transient occurrence of loose stools in rats, increased absolute and/or relative caecal weights in both rats and mice, and slightly increased serum ALP reported in rats, was concluded to be due to the loading of the large intestine of unabsorbed erythritol and its fermentation products. As such, these effects also represent physiological responses that are well documented to occur with high-dose exposure to other low molecular weight substances, often osmotically active, that undergo fermentation in the large intestine. Such physiological responses may be the result of the trophic effect on the colonic mucosa associated with the reduction in faecal pH caused by the presence of fermentation products (e.g. volatile short-chain fatty acids) (Sakata, 1987). Increased faecal bulk, probably resulting from an increase in water content in response to the presence of osmotically active substances (Leegwater *et al.*, 1974; Walker, 1978), may also partially account for the increased caecal weights in rodents associated with high-dose oral exposure to erythritol.

The reported increases in serum ALP in rats are not indicative of a systemic effect since the large intestine is known to be the main source of this enzyme in non-fasting rats (Righetti and Kaplan, 1971) and similar increases in serum ALP have been demonstrated with other osmotically active carbohydrates which reach the large intestine following ingestion at high doses (Bär *et al.*, 1995; Moser *et al.*, 1980; Schaafsma and Visser, 1980; Woutersen, 1987). As a result, the modest increases in serum ALP are considered related to caecal enlargement (Til and Modderman, 1996).

There are several lines of evidence indicating that the rodent findings of increased absolute and/or relative caecal weights, transient laxative effects/occurrence of loose stools, and increased serum ALP values represent physiological responses to the increased osmotic load of the large intestine. First, these effects occur only at high doses where there would be the potential for significant transit of unabsorbed erythritol to the large intestine. In dogs, in which metabolic studies suggest that lesser amounts of erythritol may be fermented in the colon compared with rodents (Nakayama, 1990a; Noda *et al.*, 1996), administration of erythritol in the diet at concentrations of up to 10% (Dean *et al.*, 1996) was not associated with increased caecal or colon weights. Secondly, in both rats and dogs, in which systemic exposure was attained by intravenous injection (i.e. no erythritol loading of the large intestine), caecal enlargement and increased serum ALP were not observed (Kamata, 1990a,b). Thirdly, in a comparative study (Oku and Noda, 1990a), rats administered erythritol or sorbitol at 10% in the diet were found to show laxative effects and to have increased caecal weights. However, the severity of the laxative effects and the increase in caecal weights were considerably greater in the sorbitol-treated rats. These findings were consistent with the knowledge that sorbitol is not as readily absorbed as erythritol and comparatively higher amounts of sorbitol reach the lower gut. Finally, in a study on erythritol which allowed for a period of recovery, caecal enlargement was found to be reversible (Yamamoto *et al.*, 1989). The observed reversibility of the caecal enlargement, as indicated by WHO (1987), further supports the consideration of this effect as a physiological response.

A number of other findings reported in the subchronic and chronic toxicity studies, including minor isolated changes in haematological, blood chemistry, urinalysis and organ weight parameters were of a small order of magnitude, generally failed to show a dose–response effect, were often inconsistent across sex, time and study, and often were within the normal physiological range. As a result, these changes were considered to be of spurious origin and to represent chance findings with no relationship to erythritol treatment. Some of the reported urinary enzyme changes, including the report of increased urinary excretion of NAG, also may be included in this category as the increases were minimal, often within the normal physiological range, and were never correlated with any histopathological findings or with any indication of renal disease.

In several reproductive toxicity (Tateishi *et al.*, 1989, 1992; Waalkens-Berendsen *et al.*, 1996) and teratogenicity studies (Ota *et al.*, 1990; Shimizu *et al.*, 1996; Smits-van Prooije *et al.*, 1996b) on erythritol, no effects were observed that were not already described in the subchronic studies. Erythritol was demonstrated to have no teratogenic activity or potential for reproductive toxicity. Similarly, three *in vitro* mutagenicity studies (Blijleven, 1990; Kawamura *et al.*, 1996) and a 2-year rat carcinogenicity study (Lina *et al.*, 1996) produced uniformly negative results.

The preclinical testing program on erythritol included all of the required toxicology studies that normally would be performed in support of a submission to regulatory agencies. The overall conclusion supported by the animal data was that erythritol did not directly cause any adverse effects but was associated, when administered at very high doses, with some general effects on water balance with subsequent alterations in water intake, urine volume, electrolyte excretion and faecal consistency.

Given the findings of the animal studies, the clinical studies provided information to compare the metabolism of erythritol in humans and animals,

and to assess the possibility for the development of effects associated with changes in water balance, both in normal and specific sub-populations, and under the expected use patterns. The clinical program, therefore, sought to establish the relevance of the toxicological data while demonstrating directly the safety of erythritol in humans. The clinical program for erythritol was considered to be adequate and reflected the choice of appropriate studies, populations (i.e. diabetics), and use patterns to establish the safety of a food ingredient as recommended by various experts and regulatory bodies (FDA, 1993; Forbes, 1996; Munro *et al*., 1996).

Transient minor gastrointestinal effects, consisting of loose stools, nausea, gurgling and flatulence, were reported in the clinical studies after erythritol administration, but only after large acute bolus (liquid) doses and/or administration on an empty stomach (Bornet *et al*., 1996a; Oku and Okazaki, 1996a,b; Takahashi, 1992b; Umeki, 1992). A dose of 1 g/kg body weight/day was tolerated without significant effects during a repeat-dose study which used divided daily doses incorporated into food and beverages and a 2-day, pre-dosing adaptation period (Tetzloff *et al*., 1996). The finding of a relatively higher incidence of gastrointestinal effects with acute bolus dosing in solution and/or on an empty stomach probably reflects greater passage of the osmotically active erythritol into the large intestine as a result of reduced absorption. Other low molecular weight organic compounds have also been reported to be associated with gastrointestinal effects when administered in liquid form and on an empty stomach (Corazza *et al*., 1988; Martini and Savaiano, 1988). In the repeat-dose study (Tetzloff *et al*., 1996), erythritol was incorporated into foods and beverages over the course of the day, which was considered to be more representative of the actual conditions of use.

Relative to sorbitol, erythritol produced gastrointestinal effects at much higher doses (Oku and Okazaki, 1996a,b; Takahashi, 1992a,b; Umeki, 1992). The data indicate an approximately three- to fourfold greater tolerance to erythritol compared with sorbitol with respect to the causation of gastrointestinal and laxative effects. Overall, fewer gastrointestinal effects would be anticipated with the use of erythritol compared with the use of sorbitol. In the studies focusing on gastrointestinal effects, and relative tolerance compared with sorbitol, it is important to note that erythritol was administered as a single bolus dose or as two doses in close succession, in an aqueous solution or jelly, and on an empty stomach. Based on clinical studies in which erythritol was administered with food, and under the intended conditions of use of erythritol as a food ingredient, a laxation threshold cannot be defined. As a result, the use of erythritol in foods would not be expected to cause gastrointestinal effects.

Of the three studies which specifically analysed urinary parameters (Bornet *et al*., 1996b; Noda *et al*., 1994; Tetzloff *et al*., 1996), no changes were found following single 0.3 g/kg body weight bolus doses of erythritol (Noda *et al*., 1994). When bolus 0.4 or 0.8 g/kg body weight doses of erythritol were administered in food, compared with sucrose controls, slight changes in urinary parameters (i.e. osmolarity, sodium and chloride excretion) occurred sporadically; however, over the entire study period these changes were not statistically significant. Moreover, in the repeat-dose study (Tetzloff *et al*., 1996), which used a divided daily dose of 1 g/kg body weight, no quantitative differences or differences relative to creatinine were reported. This latter study did report some statistically significant increases in urinary calcium and phosphate; however, these increases were small and within normal physiological ranges. The changes in osmolarity were attributed to the presence of erythritol itself in the urine (Tetzloff *et al*., 1996), since the increase parallelled that which would be expected based on the concentration of erythritol in the urine. Urinary volume followed a similar pattern to the electrolyte and osmolarity changes; however, no statistical differences were reported and, in fact, urine volume was lower than that occurring with sucrose in both studies in which a comparison between the two substances was made. Given the nature of the changes in the above urinary parameters, it was concluded that erythritol did not adversely affect renal capacity.

On a statistical basis, following treatment with erythritol the urinary excretion of NAG, albumin and β_2-microglobulin (Tetzloff *et al*., 1996) were increased compared with sucrose-treated controls. A critical evaluation of the urinary enzyme/protein data from the Tetzloff *et al*. (1996) study indicates that the reported increases in urinary excretion of NAG, albumin and β_2-microglobulin were not toxicologically significant. Several lines of evidence support this conclusion. First, with respect to NAG and β_2-microglobulin, the mean values for these parameters were increased approximately 25 to 30% over the sucrose-control values (Tetzloff *et al*., 1996); however, the individual data showed that there was considerable variability in the levels and that the highest values were often seen in the sucrose control group. Over the course of the study, there were no pronounced changes in the group mean values for NAG and β_2-microglobulin and the level of fluctuation seen in some individuals did not appear to be altered by treatment. Secondly, the absolute values for NAG, albumin and β_2-microglobulin remained within normal physiological ranges. Given the small magnitude of the reported statistical differences, the nature of the inter-individual variation, the finding that urinary excretion of NAG, albumin and β_2-microglobulin remained within normal physiological ranges, and noting the

lack of an effect of erythritol treatment on NAG or albumin (β_2-microglobulin was not measured) in the Bornet *et al*. (1996b) study, the changes in urinary excretion of NAG, albumin and β_2-microglobulin reported by Tetzloff *et al*. (1996) do not represent clinically significant findings. This conclusion is also consistent with the lack of histopathological effects in animals that showed changes in the same urinalysis parameters following exposure to high doses of erythritol. Based on the results of the clinical studies it is concluded that erythritol is well tolerated and not associated with any adverse effects.

Conclusions

The large body of published data supports the conclusion that the intake of erythritol would not be expected to cause adverse effects in humans under the conditions of its intended use in food. The available studies demonstrate that erythritol is readily absorbed, is not systemically metabolized, and is rapidly excreted unchanged in the urine. Moreover, erythritol occurs endogenously and naturally in the diet. Both animal toxicological studies and clinical studies have consistently demonstrated the safety of erythritol, even when consumed on a daily basis in high amounts. Based on the entire safety data package on erythritol, it is concluded that erythritol is safe for its intended use in food.

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