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Safety Assessment of Polyol Sweeteners—Some Aspects of Toxicity

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ABSTRACT

For the assessment of the safety of food additives, data from a battery of toxicity tests are required. Because in such studies the experimental animals are often exposed to extremely high doses of the test compound, pathological changes may be detected which under more physiological conditions would not be observed. As an example, results of chronic and subchronic toxicity tests are presented in which laboratory animals have been fed on diets containing high concentrations of slowly digestible carbohydrates (lactose, raw potato starch, modified starches), sugar alcohols (xylitol, sorbitol, mannitol, lactitol) or bulking agents (polydextrose). Diarrhoea, caecal enlargement, pelvic nephrocalcinosis and adrenal medullary hyperplasia were noted in some of these studies as pathological end-points. Subsequent biochemical investigations revealed the underlying mechanisms for some of these effects. These data, together with results of clinical studies, indicated that the pathological effects observed in animal tests were of no significance for man, partly because of species differences and partly because of different levels of exposure. The results of these and other studies have drawn attention to the desirability of adopting more flexible approaches to the testing requirements for certain food additives, depending on such factors as their structure, occurrence in nature, and metabolism into normal metabolites, as well as their proposed use.

The toxicological evaluation of a food additive comprises two stages. The first is the collection of relevant data. These are mainly derived from

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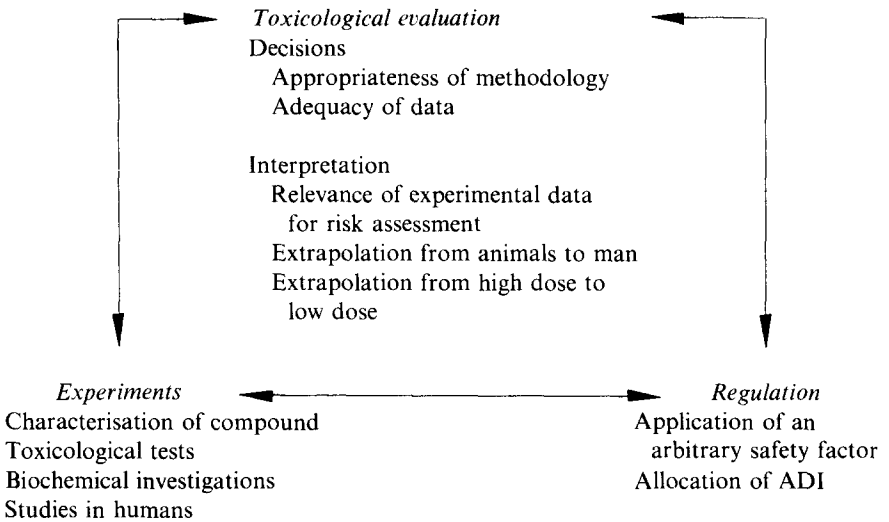

Fig. 1. Science and art of safety assessment.

experimental testing in laboratory animals but supplemented, whenever possible, by human data. The second step is the interpretation and assessment of all the data in order to decide whether the substance is suitable or not for use as a food additive (Fig. 1) (for general principles see Vettorazzi, 1980). The first step, i.e. performing toxicological tests, is usually relatively straightforward, and follows established study designs and routine procedures which will not be discussed in this article. The purpose of this paper is to illustrate the complexity of the problems which some bulk sweeteners have posed during the second interpretive and assessment phase.

Sorbitol, mannitol, xylitol, and lactitol are sugar alcohols which have a sweet taste and which may replace sucrose in certain products. The first three occur in small amounts in fruits and vegetables. Xylitol has also been identified as an intermediate product in mammalian carbohydrate metabolism. Studies in man suggested that it is produced in amounts of between 5 and 15 g daily in the human liver (for a review, see Mäkinen, 1978).

Considering the chemical structure and the physiological occurrence of xylitol and the other polyols, it was not surprising that tests *in vitro* for mutagenicity and clastogenicity have given uniformly negative results for all these compounds, and that animal tests for acute toxicity have indicated only a very low level of toxicity by all routes of

TABLE 1
Typical Effects of Chronic Consumption of Polyols and Slowly Digestible Carbohydrates in Rats and Mice

	Lactose	*Lactitol*	*Sorbitol*	*Mannitol*	*Xylitol*	*Ribose*
Reduced weight gain	+	+	+	+	+	+
Diarrhoea	+	+	+	+	+	+
Caecal enlargement	+	+	+	+	+	+
Hypercalciuria	+	+	+	+	+	+
Pelvic nephrocalcinosis	+	+	+	?	+	+
Bladder calculi (mice)	?	?	+	—	+	+
Adrenomedullary hyper-/neoplasia (rats)	+	+	+	±	+	?

administration. Conventional tests for embryo-toxicity and teratogenicity, and for adverse effects on reproduction, have also given entirely negative results. In view of these results which have consistently demonstrated the lack of toxic effects, it was somewhat surprising when, in chronic toxicity tests, it was found that the long-term ingestion of high doses of some sugar alcohols was associated not only with physiological responses to the treatment, such as transient laxation and caecal enlargement, but also with clearly adverse effects, such as the formation of urinary tract calculi and tumours of the bladder epithelium in male mice, and pelvic nephrocalcinosis and tumours of the adrenal medulla in male rats. As shown in Table 1, these effects were not specific just for xylitol and sorbitol. On the contrary, a remarkably similar spectrum of effects was observed for almost all polyols that have been subjected to relevant feeding tests, although, depending upon the maximal dose level tested and the species and strain of test animals used, they were more or less overt for the different test compounds.

Obviously it is not possible in this short article to discuss in depth the interpretation and relevance of all these findings. The following discussion is therefore mainly focused on the biological significance of the bladder calculi and tumours which were observed in chronically xylitol-fed mice. This selected example serves to illustrate the kind of problem which may occur when bulking agents or sweeteners are subjected to routine toxicity tests at levels in the diet which are not only greatly in excess of those to which man is exposed, but also nutritionally significant.

TABLE 2
Changes in the Urinary Bladder of Male Mice[a]

Group	*Stones present*	*Epithelial hyperplasia*	*Epithelial metaplasia*	*Any tumour*
C	2%	16%	0%	0%
20S	3%	13%	0%	0%
2X	2%	12%	0%	0%
10X	63%	71%	8%	15%
20X	76%	73%	16%	17%

[a] (Hunter *et al.*, 1978)

In the course of the toxicological testing of xylitol, a chronic carcinogenicity/toxicity study was conducted in which groups of 100 male and 100 female CFLP Swiss albino mice were exposed to diets containing 2, 10, or 20% xylitol or 20% sucrose. Controls were fed on a control diet of which 20% was native rice starch. In the 20% dose group, the minimum achieved xylitol intake over the entire treatment period was 17·0 and 19·6 $g\,kg^{-1}\,day^{-1}$ for males and females, respectively. At the end of the study, macroscopic and microscopic examination of the animals revealed a high incidence of bladder stones and a low incidence of neoplasms of the bladder epithelium in the male mice of the 10 and 20% xylitol groups. No similar effects were observed in female animals (Table 2). On chemical analysis, the stones were found to consist of calcium oxalate (Hunter *et al.*, 1978).

At the time of termination of the study, it was already known that the presence of stones predisposes non-specifically to bladder tumour development in mice (Ball *et al.*, 1964). Consistent with this was the observation that neither stones nor bladder tumours were seen in males in response to the 2% xylitol diet, that no stones or tumours were seen in

TABLE 3
Calculation of Acceptable Daily Intakes

$$\frac{\text{NOEL}}{10 \times 10} = \text{ADI}$$

Example: no effect level (NOEL), 2% Xylitol ≅ 1·7 $g\,kg^{-1}\,day^{-1}$;
acceptable daily intake (ADI), 0·017 $g\,kg^{-1}\,day^{-1}$;
a 79 kg adult could consume 1·19 $g\,day^{-1}$.

females at any dietary concentration of xylitol, and that, with only one exception, all the mice that developed bladder tumours also had bladder stones at the time of necropsy. In view of these facts, and considering the negative results of the *in vitro* mutagenicity test, it was therefore concluded that the observed tumours of the bladder epithelium were secondary to the presence of vesical calculi and that xylitol did not exert a direct genotoxic action. Regulatory action against xylitol on the basis of suspected carcinogenicity was therefore not justified. Nevertheless, questions regarding the relevance for human safety of the bladder stone formation remained unanswered at first, and caused some concern.

With such results of a chronic carcinogenicity/toxicity study available and analysed by toxicologists and statisticians, what happens next? In the case of a non-nutrient food additive, the regulatory toxicologist would normally determine a 'no effect' dose level for the most sensitive species used in a lifetime study, and apply an arbitrary safety factor in order to estimate a dose which may be safely consumed by man daily for a lifetime (Table 3). Usually a 100-fold safety factor is applied, which consists of a 10-fold factor for differences between the test species and man, and a 10-fold factor to account for inter-individual differences of susceptibility. Obviously, this concept is not applicable for bulk sweeteners and bulking agents which, under their intended conditions of use, could be included in the daily food in amounts exceeding 1 % of the diet. In recognition of this problem, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has therefore concluded in its 17th Report that lower safety factors may be applied for substances that are normal constituents of the human diet or normal intermediary metabolites, for substances that are not absorbed from the gastrointestinal tract, for substances that in humans are converted by metabolism or by digestion to a normal constituent of the diet, or for substances for which sufficient data on human tolerance are available (WHO/FAO, 1974).

Although it is officially accepted that a 100-fold safety factor cannot be applied to certain food ingredients, there is often a tendency, based on the assumption that negative results will be obtained, to raise dosage in chronic bioassays to unreasonably high levels in order to obtain as high an ADI for the test compound as possible. As a consequence, there arises the serious risk of metabolic overload, which should always be expected when nutritive materials are fed in excessively high amounts. Moreover, chronic dietary imbalance may result from the inclusion of high levels of either nutritive or undigestible materials. The effects of metabolic

TABLE 4
Urinary Calcium Excretion

Treatment	*Calcium (μg per 24 h)*
Control	249 ± 143 (34)
2·5% Xylitol	298 ± 239 (36)
5% Xylitol	605 ± 330 (35)
10% Xylitol	873 ± 449 (36)
20% Xylitol	1 818 ± 799 (36)

overload and/or chronic dietary imbalance need then to be distinguished from toxic effects which are directly attributed to exposure to the test compounds. Concern among regulators and consumers about albeit non-specific effects of metabolic overload or chronic nutritional disturbance commonly lead to demands for studies of the mechanism involved and in delays before permission is granted for the use of much lower levels of substances in food.

In the case of xylitol, regulators were faced with non-specific high dosage effects both in mice (bladder calculi and tumours) and in rats (adrenomedullary proliferations but no bladder calculi). The Joint FAO/WHO Expert Committee on Food Additives decided at its twenty-second meeting (WHO/FAO, 1978) that the available data base was insufficient for a toxicological evaluation and that further studies should be carried out. Regarding the formation of bladder stones in mice, it was recommended that the urinary chemistry of human beings to whom high levels of xylitol are administered should be investigated.

In response to these recommendations, a series of short-term studies was undertaken which elucidated the reasons for the calcium oxalate stone formation in mice. The results of these so far unpublished studies, together with data from clinical trials, demonstrated that the ingestion of xylitol is not associated with an increased risk of bladder stone formation in man. The scientific basis for this conclusion is as follows.

The risk of calcium oxalate bladder stone formation is mainly dependent on three factors: the urinary concentration of oxalate, the urinary concentration of calcium, and the urinary pH. The urinary concentration of other ions such as magnesium, phosphate, urate, and citrate may also play a rôle in stone formation, but these factors are certainly less important. Investigations on the effect of xylitol and sorbitol consumption on these risk factors gave the following results: in mice and

TABLE 5
Incidence of Urolithiasis in Male Fü-Albino Mice

Treatment (20% dose 24 weeks)	*Fü-Albino mice, terminal kill*	
	Nephrocalcinosis (degrees 2 and 3)	*Bladder calculi (degrees 2–4)*
Starch	3/24	0/24
Xylitol	9/25	10/25
Sorbitol	6/21	3/21
Ribose	2/24	2/24

rats, the urinary calcium excretion was increased by a factor of between 7- and 10-fold following the administration of xylitol and sorbitol at the 20% dose level. This hypercalciuria was dose-dependent (Table 4) and resulted from increased calcium absorption from the gut. Since this effect was seen not only after ingestion of xylitol but also after the consumption of other polyols (e.g. sorbitol) and slowly digested carbohydrates (e.g. lactose), it was assumed that the exposure of mice to sugar alcohols other than xylitol might also increase the risk of bladder stone formation. At least for sorbitol, and perhaps also for ribose, this was indeed seen, although the effect was less pronounced than for xylitol (Table 5). From this observation, which was confirmed in subsequent tests, it was concluded that the polyol-induced absorptive hypercalciuria contributed to the formation of uroliths but that, in the case of xylitol, an additional factor further enhanced the risk of stone formation.

The possibility of a partial conversion of xylitol into oxalate was suggested in the early seventies when the occurrence of renocerebral oxalosis in deceased intensive care patients was attributed to the prior parenteral use of xylitol. Subsequent biochemical experiments in xylitol-fed rabbits and rats, and with liver cell homogenates derived from rats, gave somewhat conflicting results. However, more consistent results were obtained in mice. In this species, the dietary administration of xylitol at the 20% dose level significantly increased the urinary excretion of oxalate and of glycollate, a metabolic precursor of oxalate (Table 6). The concomitant increase of both glycollate and oxalate is consistent with a proposed minor pathway of xylitol catabolism leading via xylulose-1-phosphate to glycolaldehyde, glycollate and finally oxalate (James *et al.*, 1982).

TABLE 6
Urinary Excretion of Glycollate and Oxalate in Two Studies on Male Fü-Albino Mice

Treatment (20% dose)	*Glycollate (mg per 24 h)*	*Oxalate (mg per 24 h)*
Experiment I		
Control	0·07 ± 0·02 (18)	0·20 ± 0·06 (22)
Xylitol	0·12 ± 0·08 (15)***	0·28 ± 0·06 (22)***
Sorbitol	0·06 ± 0·02 (18)	0·20 ± 0·06 (20)
Experiment II		
Xylitol	0·12 ± 0·05 (45)***	0·30 ± 0·07 (46)***
Sorbitol	0·06 ± 0·02 (35)	0·20 ± 0·06 (32)

*** $p < 0·01$.

Taken together, these investigations demonstrated that xylitol adversely affected two risk factors of urolithiasis at the same time, namely the urinary concentration of calcium and the urinary excretion of oxalate. As a result, xylitol was more active in inducing bladder stones than sorbitol, which had a similar effect on calcium excretion but which did not increase the urinary oxalate levels.

Knowledge of these results led to the next question: whether similar changes in urine composition might also be observed in humans following the consumption of large doses of xylitol. Earlier, a number of tolerance studies on healthy volunteers suggested that this was not the case (Mäkinen, 1978; Raunhardt & Ritzel, 1981). However, it was felt that minor effects on the concentration of calcium and oxalate in the urine occurring during only short periods of the day may have been overlooked in studies where analyses were confined to 24-h urine samples. Two additional studies were therefore carried out.

In the first study, 12 adult human volunteers consumed xylitol or sucrose supplements during periods of 7 days, together with a self-selected diet. For both supplements the dose was 0·3 g per kg bodyweight on the first day, 0·6 g per kg bodyweight on the second day and 1·0 g per kg bodyweight on the subsequent 5 days. Urine was collected at 2-h intervals during the working day and at less frequent intervals at home and during the night. The analysis of the samples showed that, in comparison with sucrose, xylitol had no effect on the 24-h calcium excretion and also no obvious effect on the diurnal pattern of calcium

TABLE 7
Conversion of Ingested Xylitol Into Urinary Glycollate and Oxalate

Species	*Number of subjects*	*Total dose ingested*	*Mean excretion in % of ingested xylitol (w/w)*	
			Glycollate	*Oxalate*
Mouse	36	$16{\cdot}8\,g\,kg^{-1}\,day^{-1}$	0·005 4	0·002 8
Man	12	$1{\cdot}0\,g\,kg^{-1}\,day^{-1}$	0·007 4	0·004 8
Man	4	$0{\cdot}013\,9\,g\,kg^{-1}$	0·137 7	0·007 3

excretion. With respect to urinary oxalate, a small but significant increase was observed for the 24-h values. However, this increase (8·3% on average) was very small compared with variations of the urinary oxalate excretion due to the dietary composition of the self-selected diet, and urine portions with elevated oxalate concentrations occurred with similar frequency during both treatment periods.

In order further to investigate the conversion of xylitol to oxalate, a second study was carried out in which four human volunteers ingested approximately 1 g of ^{13}C-labelled xylitol. GC/MS analysis of the urine showed that only about 0·07 mg of labelled oxalate appeared in the urine during a 6-h period following the ingestion of the compound. After a similar dose of ^{13}C-labelled glucose, no labelled oxalate appeared in the urine of the same subjects.

From the data obtained in this and the previous study, the conversion of xylitol to oxalate was calculated. It was found that, on average, between 0·005 and 0·007% of the administered xylitol load was recovered as urinary oxalate (Table 7). These figures show that xylitol is even less effective as an oxalate precursor than glycine, for which a conversion factor of 0·03–0·05% was established (Gibbs & Watts, 1969; Tschöpe *et al.*, 1983). It is therefore not surprising that in comparison with xylitol, the ingestion of certain dietary oxalate precursors or of dietary oxalate itself contributes far more to the total daily oxalate excretion.

On the basis of the reported data it may, therefore, safely be concluded that the consumption of xylitol by humans, even at relatively high dose levels, carries no risk of increased calcium oxalate bladder stone formation and therefore no risk of bladder cancer. In its 26th and 27th Reports, the Joint FAO/WHO Expert Committee on Food Additives (WHO/FAO, 1982, 1983) reached the same conclusion and allocated an ADI 'not specified' to xylitol, sorbitol and lactitol. This classification implies that

the members of JECFA felt it unnecessary to limit the exposure of humans to these various polyols.

Regarding the increased occurrence of adrenal medullary proliferative disease in rats (*vide supra*), the Committee considered this to be non-specific in nature, and concluded that it was probably related to metabolic and physiological disturbances to gross dietary imbalance (WHO/FAO, 1983). In this respect, experimental data have recently been reported which suggest that the observed adrenal medullary hyperplasia and neoplasia seen not only in polyol-treated rats but also in lactose-treated rats, are secondary to the increased calcium absorption from the gut (Roe and Bär, 1985).

The data presented in this paper have centered on the effects of xylitol in experimental animals and in man. The example of xylitol illustrates the complex issues which are faced by toxicologists and regulators in the extrapolation of animal data to man. Non-specific adverse effects are likely to be encountered wherever food additives are fed at high dose levels to laboratory animals. Similar effects do not occur in man, partly because metabolic pathways are quantitatively different and partly because humans are not exposed to the extremely high dose levels associated with adverse effects in animals. Typical problems in this respect were also encountered, for example, in the toxicological evaluation of polydextrose (Schach von Wittenau, 1981; WHO/FAO, 1980) and of some modified starches (Hodgkinson *et al.*, 1982; WHO/FAO, 1976, 1982).

The conclusion is that, in the case of food additives which are intended to replace normal food ingredients in substantial amounts, there is a particular need to investigate the metabolic properties of the material and to conduct clinical studies in man. In this respect, an important aim of the animal studies is to indicate the direction of changes to look for in man. In any case, the experimental approaches must be flexible and specifically adjusted to the physiological properties of the test compound.

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