A Review and Biological Risk Assessment of Sodium Saccharin

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Dietary sodium saccharin is associated with bladder tumors when fed at high levels to the male rat. Under these conditions urinary pH, sodium concentration, and volume are elevated and proliferative changes are present in the urothelium. Extensive epidemiological studies have shown that saccharin does not increase the risk of bladder cancer in humans and laboratory investigations have shown that sodium saccharin is not mutagenic and does not bind to DNA. Recent research indicates that the urothelium in male rats is damaged under conditions of high urinary pH and sodium levels by a mechanism that involves α₂-globulin and possibly silicate crystalluria. These studies and their implications for human health risk are reviewed.

INTRODUCTION

Saccharin has been employed extensively as a sweetener in foods and beverages for more than eight decades. During this time it has generally been accepted that this additive posed no health risks, although, as summarized by Arnold (1984), the history of saccharin use has not been free of controversy. During the 1970s carcinogenicity studies in the rat using two-generation protocols showed that when fed for long periods at high dietary concentrations sodium saccharin was associated with the development of bladder tumors. Since then the safety of saccharin has been intensively studied to determine the nature of the bladder changes that occur in the rat, the mechanism underlying the changes, and the significance of these findings for humans.

Saccharin is almost unique among food additives since extensive biochemical, physiological, and toxicological studies in animals are matched by equally comprehensive epidemiology studies in man. Epidemiological studies support the conclusion that saccharin does not pose a risk of bladder cancer in humans. Animal studies have shown that the development of bladder tumors after sodium saccharin is fed is unique to the rat and that tumors do not occur after long-term feeding of sodium saccharin to mice, hamsters, or monkeys. Extensive laboratory investigations conducted to determine the mechanism(s) underlying the bladder tumors that occur in the rat fed sodium saccharin suggest a phenomenon of considerable complexity. Long-term studies of sodium saccharin have shown that bladder tumors occur predominantly in the male rat and only when this sweetener is fed at high dietary levels beginning at or prior to birth (Schoenig et al., 1985). Studies of sodium saccharin and the sodium
salts of other weak to moderate organic acids in bladder tumor promotion paradigms have shown that the feeding of high dietary levels of these substances to the rat is associated with increased urine volume, high urinary sodium concentration, and high urinary pH. Modification of these urinary biochemical and physiological parameters by feeding urine acidifying agents or diets that result in acid urine eliminates the bladder tumor response. The development of bladder tumors in the rat is, therefore, not only predominant in the male rat but dependent on specific physiological preconditions within that sex and species.

Many studies have been conducted to determine the role of established carcinogenic mechanisms in the bladder cancer associated with sodium saccharin. The results of these studies have generally been negative and have shed little light on sodium saccharin tumorigenicity. Saccharin is neither mutagenic nor genotoxic and does not bind to DNA. It is not metabolized and does not accumulate in tissues. It does not cause malignant transformation of cultured bladder cells, nor does it activate ras oncogenes. Saccharin does not bind to bladder epithelial cell membranes nor to epithelial growth factor receptors and the tumorigenesis cannot be explained on the basis of impurities or contaminants.

The following risk assessment has been conducted, taking into account the vast data base available on saccharin. It appears obvious from the information presently available that a quantitative risk assessment of saccharin based on mathematical "one-hit" or linearized nontreshold multistage models is lacking in scientific support or justification. The considerable body of evidence that supports a biologically based risk assessment is presented.

METABOLISM/PHARMACOKINETICS

Extensive studies of the disposition of saccharin have been conducted in laboratory animals and man. These investigations have been thoroughly reviewed by Renwick (1985, 1986). Studies of sodium saccharin, in brief, have shown that in the rat and man following oral administration saccharin is slowly absorbed from the gastrointestinal tract and rapidly eliminated unchanged in the urine largely by renal tubular secretion. Saccharin is not metabolized and in the rat it does not accumulate in any tissues including the bladder. At high dosage levels, i.e., dietary concentrations of sodium saccharin greater than 5%, renal tubular secretory mechanisms become saturated in the rat but no saturation of renal tubular secretions has been observed after oral or intravenous doses of sodium saccharin in humans. It has been concluded that the rat is a reasonable model for the metabolism and pharmacokinetics of saccharin over the range of intakes that occur in the general use of this additive by humans.

URINARY PARAMETERS

The bladder of the rat is the only target organ for sodium saccharin-induced tumor formation. Statistically significant, dose-related changes in bladder weight and mineral content, and in urine volume and composition have been observed in rats fed sodium saccharin (Schoenig and Anderson, 1985). These nonneoplastic responses have been found to precede, and to occur in association with, tumor development. Much attention has been focused on these nonneoplastic responses in an attempt to gain a better understanding of the mechanisms of sodium saccharin tumorigenesis.
Studies of bladder tumor promotion in the rat by sodium salts of weak to moderately strong organic acids have demonstrated that the effects of high dietary concentrations of these sodium salts on bladder epithelium are associated with increased urinary concentrations of Na⁺ and high pH (Fukushima et al., 1986b; Shibata et al., 1989b; Cohen et al., 1991b). The importance of high urinary pH is illustrated by the findings with sodium hippurate. Unlike other sodium salts of organic acids sodium hippurate did not increase urine pH and did not promote bladder tumors in male rats in the two-stage bladder tumor promotion model (Ito et al., 1983; Fukushima et al., 1983a; Fukushima et al., 1986d). Furthermore, sodium hippurate did not cause bladder tumors in male rats in a long-term bioassay (Schoenig et al., 1985). Numerous bladder tumor promotion studies have shown that both increased urinary Na⁺ and a high pH are essential for the induction of bladder tumors by the sodium salts of organic acids, including saccharin.

Ingestion of a high sodium level by the rat leads to polydypsia, high urine volume, and as a consequence low urine osmolality. Anderson et al. (1987) and Anderson (1988) have suggested on the basis of studies with rats fed 7.5% sodium saccharin that the bladder tumorigenicity of this material in the male rat may be a consequence of increased urine volume and bladder distension. Renwick and Sims (1983) have also demonstrated increased water intake, urine volume, and urine volume per micturition in male rats fed 7.5% sodium saccharin and suggest that the bladder distension associated with these saccharin effects may be a factor in the development of bladder tumors. It has been suggested that the increased ratio of urine volume to bladder mass observed may cause a thinning of the protective lining of the urothelium, resulting in an increase in the surface area through which potential carcinogens may gain access to the underlying cells (Schoenig and Anderson, 1985).

It is possible that bladder distension associated with the excretion of large volumes of urine with low osmolality and increased Na⁺ concentration may be a factor in the pathogenesis of bladder tumors in rats fed sodium saccharin. Studies of the diuretics furosemide and acetazolamide, however, have shown that increased urine volume alone does not cause bladder cancer development (Shibata et al., 1989a; Fukushima et al., 1983a). The physiological changes in the urine in these studies were not identical to those caused by sodium saccharin; thus a role for bladder distension in the induction of bladder tumors by sodium saccharin cannot be totally excluded.

Changes in bladder permeability brought about by distension, changes in urine osmolality, or other factors related to increased urinary concentrations of saccharin could be potentially important in the mechanism of sodium saccharin effects on the bladder. Such changes in permeability were proposed by Lawson (1982) and Imaiida et al. (1983). However, recent studies of permeability and bioelectric processes in the rat bladder in vitro have led to the conclusion that neither sodium saccharin feeding nor exposure of the bladder to urine from sodium saccharin-fed animals affects the permeability of the rat bladder (Gatzy et al., 1989).

SALT FORM

The cation associated with the saccharin ion appears to be critical to the pathogenesis of bladder lesions in the rat. In weanling rats fed sodium or potassium saccharin at 5% of the diet for 10 weeks, urine volume was increased, pH was high, osmolality was decreased, and urothelial proliferation was present as determined by light and electron
microscopy and thymidine labeling. When rats were fed equivalent dietary levels of calcium or acid saccharin, urinary pH was decreased, urine volume was unaffected, osmolality was slightly increased, and no increase in thymidine labeling was observed. Two of twelve rats fed calcium saccharin had simple hyperplasia in the urothelium but hyperplasia was not seen in the rats fed acid saccharin (Hasegawa and Cohen, 1986). Urinary saccharin concentrations were not affected by the salt form of the saccharin fed. It has also been noted that acid saccharin does not induce bladder tumors in the two-stage bladder tumor promotion model (West et al., 1986; Cohen et al., 1991b). These studies demonstrated the critical role that the cation plays in the effects of saccharin salts on the bladder.

The finding that the sodium, potassium, calcium, and acid forms of saccharin differ in the extent to which they produce epithelial proliferation in the bladder of the rat and that these differences were independent of urinary saccharin concentration suggested the possibility that different physicochemical forms of the saccharinate ion might be present in urine. In order to explore this possibility, alterations in the NMR spectra of the saccharinate ion in the presence of varying concentrations of hydrogen, potassium, sodium, calcium, magnesium, bicarbonate, and urate were studied. None of these ions at physiological levels significantly altered the electronic structure of the saccharin molecule. It was concluded that differences in the mitogenic response of the rat bladder to different salt forms of saccharin could not be explained on this basis (Williamson et al., 1987).

**DIETARY EFFECTS ON URINE COMPOSITION AND BLADDER RESPONSE**

It is well known that diet is a major factor affecting urinary composition in the rat. All of the long-term rat studies with positive bladder tumor findings were conducted using cereal-based commercial diets. Urine pH is significantly lower in rats fed semisynthetic diets compared to those fed commercial, cereal-based diets (Fisher et al., 1989; Garland et al., 1989; DeGroot et al., 1988). Urothelial changes as assessed by labeling index, histopathology, and SEM were greater in rats fed cereal-based diets containing sodium saccharin than in those fed a semisynthetic diet containing sodium saccharin (Garland et al., 1989). Similarly, in a study in which rats were fed either a purified or a cereal-based diet containing MSG, hyperplastic changes in the urothelium were significant only in those fed the cereal-based diets (DeGroot et al., 1988). It was concluded that the hyperplastic effect of MSG was due to the exposure of the urothelium to alkaline urine. Feeding a cereal-based diet containing MSG plus NH4Cl to the rat decreased urinary pH and prevented hyperplasia, while feeding a cereal-based diet containing MSG plus NaHCO3 increased urinary pH and severity of the urothelial hyperplasia (DeGroot et al., 1988). Tumor promotion experiments have also demonstrated the importance of urinary pH in the bladder tumor response to sodium saccharin. Cohen et al. (1991b) demonstrated that the bladder tumor response in rats fed sodium saccharin after initiation with FANFT did not occur when NH4Cl was added.

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1 Abbreviations used: MSG, monosodium glutamate; BBN, N'-butyl-N-(4-hydroxybutyl)nitrosamine; FANFT, N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; TES, tetraethyl orthosilicate; ENS, ethylsulfonylnaphtalene-1-sulfonamide; AAF, 2-arylaminofluorene; EM, electron microscopy; SEM, scanning electron microscopy; IRDC, International Research and Development Corp.; IARC, International Agency for Research in Cancer; NOEL, no-observable-effect level.
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added to the diet. Similarly, Imaida and Wang (1986) and Okamura et al. (1990) found that sodium saccharin did not promote bladder tumors in FANFr-initiated rats when fed the semisynthetic AIN-76A diet which is associated with an acidic urine, whereas bladder tumor promotion and a high urinary pH level were observed in rats fed sodium saccharin in cereal-based diets.

A further dietary factor which may modulate the tumorigenic potential of bladder tumor promoters in the rat is silica content. Cereal grains that are widely used in formulating rodent feeds are a major source of dietary silica. Cohen et al. (1989) observed that male weanling F344 rats fed 7.5% sodium saccharin in the diet had fewer crystals in the urine than rats fed a basal diet without sodium saccharin, but of those crystals present 50–70% contained silicon and were jagged in nature. Silicate crystals were also observed on and penetrating the urothelium, causing urothelial injury. Silicon-containing crystals were rare in the urine of rats fed the basal diet without saccharin.

CRYSTALLURIA AND BLADDER IRRITATION

Evidence that bladder changes and signs of irritation are present in rats fed sodium saccharin may be adduced from several studies of this sweetener. Anderson (1987) reported that 50% of animals fed a diet containing 7.5% sodium saccharin had hemoglobin in their urine during the first week of treatment. Hemoglobin was not present in the urine of controls. Hematuria has also been a frequent finding in long-term studies of sodium saccharin. In some of these studies hematuria was found to be associated with a developing bladder tumor. In the most recent long-term study of sodium saccharin conducted by IRDC, however, there was no correlation between the presence of hematuria in the test animals and the subsequent development of bladder tumors in the same animals (Schoenig et al., 1985). Thorough studies of the upper renal tract of rats fed sodium saccharin did not reveal pathological changes that would explain the hematuria. Examination by SEM of the bladder of rats fed sodium saccharin has shown penetration of the urothelium by crystalline material and the presence of red blood cells exuding from the damaged area (Cohen et al., 1991a). The presence of jagged and sharp crystals may explain the hematuria reported in rats fed sodium saccharin that do not have bladder tumors.

Arnold et al. (1980) noted that rats fed 5% sodium saccharin in the diet had a milky flocculent precipitate in their urine which was more pronounced in male than female rats. Subsequent analysis of this precipitate demonstrated the presence of saccharin and protein. Anderson reported a similar flocculent precipitate in the urine of male rats fed a diet containing 7.5% sodium saccharin (R. L. Anderson, personal communication). Cohen observed a fluffy precipitate and free silicate crystals in the urine of male rats fed sodium saccharin. Analysis of the precipitate and crystals revealed the presence of protein silicate complexes, the protein of which had the same peak by gel filtration as the male rat-specific protein α₂-globulin (Cohen et al., 1991a).

The presence of a precipitate and crystals containing protein that is primarily α₂-globulin in the urine of rats fed sodium saccharin may explain the sex and species specificity of the bladder tumors associated with this sweetener and also the role of the urine physiological changes that are present when tumors develop in promotion studies with sodium saccharin and other sodium salts. The formation of silicate crystals and precipitate is enhanced by high urinary sodium concentrations and a pH greater
than 6.5. An interaction between saccharin and α2u-globulin is also suggested by the finding that the incidence of aging male rat nephropathy, a disease related to the tubular retention of this protein, is reduced in rats fed sodium saccharin (Murasaki et al., 1982; Schoenig et al., 1985; Johansson et al., 1986). Silicate crystals have not been found in the urine of mice or the urine of female rats fed sodium saccharin which is in keeping with the absence of α2u-globulin in mice and the relative paucity of this protein in the urine of female rats (S. M. Cohen, personal communication). Silicon also binds to urinary albumin, although to a lesser extent than to α2u-globulin (S. M. Cohen, personal communication). The slight albumin binding of silicon and the resultant crystal or precipitate formation may explain the very low incidence of bladder tumors observed in female rats fed sodium saccharin.

Emerick and Lu have reported a series of studies in which TES was used as a model substance to produce silica urolithiasis and associated urothelial hyperplasia in the rat (Emerick, 1984; Emerick and Lu, 1987). E. M. Garland (personal communication) found that the morphological changes in the bladder of rats fed TES (i.e., erosion of mucosa, submucosal edema, and simple hyperplasia) resemble the bladder changes produced by high dietary levels of sodium saccharin.

Cohen et al. (1991a) have offered an hypothesis for events that occur in rats fed sodium saccharin: When this sweetener is fed at high dietary levels to male rats the concentrations of urinary sodium and saccharinate ion are high and the pH is above 6.5. Saccharin and α2u-globulin bind to provide a nidus for the formation of silicon-containing crystals and precipitate. The precipitate enters urothelial cells and is cytotoxic. The precipitate and silicate crystals also act as microabrasives, irritating the mucosa and causing focal necrosis. The loss of urothelial cells is followed by regenerative hyperplasia and the increased cell proliferation, sustained over the rats' lifetimes, provides the basis for the development of urinary bladder tumors.

This hypothesis is consistent with the established correlation between urinary bladder calculi or crystalluria and bladder tumors in the rat. Papillomatosis and/or tumor development associated with calculi or crystals has been demonstrated in rats fed numerous chemicals (Clayson, 1974). It is generally accepted that bladder changes in animals with calculi or crystalluria are the result of physical rather than chemical effects on the bladder urothelium. For example, reducing urinary pH in mice fed ENS by feeding NH4Cl prevented calculi formation, reduced crystalluria and inhibited the urothelial changes observed when control animals were fed this chemical alone, even though urinary concentration and metabolism of ENS were unchanged (Flaks et al., 1973, Levi et al., 1971).

ANALYSIS OF DOSE-RESPONSE FOR NONNEOPLASTIC CHANGES

The dose-response relationship between sodium saccharin consumption and non-neoplastic changes in the bladder has been characterized in a feeding study in which rats received sodium saccharin at levels of 1, 3, 5, and 7.5% in the diet (Schoenig and Anderson, 1985). Dose-response relationships were evaluated for various parameters including fecal water content, relative cecal weight, urine osmolality, urine volume, urine Na+ concentration, bladder weight, and bladder tissue mineral content. Similarly, Murasaki and Cohen (1981) studied the light microscopic and EM changes in the bladder of rats fed sodium saccharin at dietary concentrations between 0.1 and 5.0%. The steep dose-response curves over a narrow range of dose levels above 1% reported
for both physiological changes in the urine and morphological changes in the urothelium provide strong evidence that these phenomena are interrelated and share a common threshold at a sodium saccharin dietary concentration between 1 and 3%.

GENETIC ALTERATIONS

The classical paradigm for chemical carcinogens involves metabolic activation to reactive electrophiles that react with DNA and induce genetic changes. Saccharin is one of a number of chemicals associated with tumor development that are not genotoxic (Tennant, 1987; Williams, 1987). Saccharin is not an electrophile; it is not metabolized to a reactive electrophile (IARC, 1980; Sweatman and Renwick, 1979); and it does not react with DNA (Lutz and Schlatter, 1977). Sodium saccharin is not active in most in vitro or short-term in vivo tests for mutagenicity or genotoxicity (IARC, 1980; IARC, 1982; Ashby, 1985).

BLADDER TUMOR PROMOTION STUDIES

Numerous tumor promotion studies have been carried out in which rats were treated with a bladder cancer initiator such as BBN or FANFT for a short period, followed by longer term treatment with sodium saccharin. Sodium salts of other weak to moderately strong organic acids, as well as the organic acids themselves, have also been assessed for bladder tumor promoting activity. These studies have shown that the sodium salts of a number of such acids are promoters of bladder cancer in the rat, whereas the acids per se do not promote bladder tumors (Table 1).

The differences in promoting activities observed between organic acids and their sodium salts appear to be due to a nonspecific factor(s) unrelated to the urine concentration of the parent organic molecule. Urinary concentrations of ascorbic acid (Fukushima et al., 1986c; Shibata et al., 1989b,c) and saccharin (Hasegawa and Cohen, 1986) did not differ when equivalent amounts of either the sodium salts or the acid forms of these respective materials were fed to rats under conditions that yield positive results for the sodium salts in bladder tumor promotion studies. Common effects of the sodium salts are increased urine pH and Na⁺ concentrations and both appear to play a critical role in tumor promotion. The parent acids cause a decrease in urine pH and do not affect Na⁺ concentration. The role of urine pH and Na⁺ concentration in bladder tumor promotion has been studied by Fukushima et al. (1988b) using rats initiated with BBN and subsequently fed diets containing 3% sodium bicarbonate or 1% sodium chloride, or a basal diet. Urinary Na⁺ concentrations in the group fed sodium chloride were as high as or higher than those in the group fed sodium bicarbonate. Urinary pH was elevated and the incidence of bladder tumors or urothelial hyperplasia significantly increased compared to controls only in the group fed sodium bicarbonate. Other studies have also demonstrated that both elevated urine Na⁺ concentration and elevated urine pH are required for positive responses in the rat bladder tumor two-stage model (Fukushima et al., 1987; Uwagawa et al., 1988; Kitahori et al., 1988; Fukushima et al., 1989).

Although elevated urine pH and Na⁺ concentration are factors in the bladder tumor promotion syndrome, they may not be effective as the sole stimuli for bladder tumor formation. In a long-term feeding study in which NaHCO₃ was fed to rats via the diet...
TABLE I


effect of weak or moderate organic acids or their sodium salts on bladder preneoplastic changes or tumor promotion

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Fukushima et al., 1986b,c

for 2 years urinary pH and Na⁺ levels were elevated but there was no increase in the incidence of preneoplastic or neoplastic urothelial lesions (Fukushima et al., 1989).

Preneoplastic and neoplastic changes occur in the bladder of rats fed sodium saccharin in promotion studies over the same high dose range that produces these changes in carcinogenicity bioassays. The changes in urine physiology (i.e., elevated sodium excretion, high pH, decreased osmolality, and increased volume), some of which have been shown to be critical for bladder tumor promotion, also occur in rats over the same dose range. Current evidence points to a secondary mechanism underlying the development of bladder tumors in both promotion and cancer studies. Bladder tumor promotion studies involve the administration of potent carcinogens as initiators and there is a shorter latency period than in long-term bioassays. Such studies therefore have little relevance in terms of the quantitative assessment of human risk. They are valuable, however, for the information they provide on carcinogenic mechanisms.

Long-term bioassays in mice have shown that sodium saccharin is not carcinogenic in this species (Roe et al., 1970; NIH Japan, 1973). A recent bladder tumor promotion study of sodium saccharin in mice reported by Frederick et al. (1989) was also negative. Mice initiated with AAF were fed sodium saccharin at dietary concentrations up to 5% for 132 weeks. Sodium saccharin feeding decreased mortality and the onset of lymphomas but did not increase the incidence of bladder or other tumors.

**Long-term bioassays of sodium saccharin**

Numerous single generation animal feeding studies have been carried out to assess the long-term effects of saccharin consumption. These studies have been summarized
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by Cranmer (1980). With the exception of the first generation of the two-generation single-dose carcinogenicity study of sodium saccharin conducted by Arnold et al. (1980), the early one-generation feeding studies did not serve to implicate sodium saccharin as a bladder carcinogen in the rat or other species.

Four studies were conducted in rats in which saccharin was fed to the F₀ generation and to the F₁ generation in utero, from birth, and subsequently for life (Taylor et al., 1980; Tisdal et al., 1974; Arnold et al., 1980; Schoenig et al., 1985). These studies were uniformly positive with respect to bladder tumorigenesis in male rats of the second generation. The positive results of the two-generation studies contrast with the negative results obtained in most of the single-generation studies. Since the differences between these studies were the exposure of the F₀ generation and the exposure of the F₁ generation in utero and from birth, these factors may have contributed to the carcinogenic response.

The importance of the time at which sodium saccharin feeding was initiated was investigated in the large bioassay conducted by IRDC (Schoenig et al., 1985). The IRDC study was the most rigorous of the long-term bioassays of sodium saccharin, employing over 2000 second-generation male rats, seven dose levels (including controls), and an unbalanced study design in which group sizes were large at lower dose levels in order to achieve greater statistical power. This study was designed so as to determine the slope of the dose–response curve for tumors and physiological changes and to determine whether the data were appropriate for use in quantitative risk assessment. One additional group of rats was exposed to sodium saccharin only during gestation via dams fed 5% sodium saccharin, while another group was exposed beginning at birth by feeding nursing dams 5% sodium saccharin and weaning the offspring from this group onto a diet containing 5% sodium saccharin that was fed for 30 months.

The IRDC study clearly demonstrated the slope of the dose–response curve and the presence of a no-effect level. Compound-related increases in the incidence of transitional-cell papillomas and/or carcinomas were present at the 4.0, 5.0, 6.25, and 7.5% levels. Slight but not statistically significant increases in transitional-cell carcinomas or papillomas were observed at the 3.0% level. Combined benign and malignant bladder tumors were significantly increased at the 3% sodium saccharin level and the incidence steadily increased with a steep dose–response curve up to the 7.5% sodium saccharin feeding level. There were no compound-related effects at the 1.0% level. The incidence of transitional-cell carcinomas was significantly increased \( (P < 0.01) \) in the group fed 5.0% sodium saccharin from birth but not in the group fed sodium saccharin only during gestation.

The IRDC study provides a definitive dose–response curve for bladder tumors in the male rat fed sodium saccharin. The curve is very steep with no effect at the 1% dietary level and an equivocal effect at 3%. The dose–response range for bladder tumors corresponds to the dose range for changes in urinary physiological parameters discussed earlier. Other dose-related changes including increased bladder weights, mineralization of the kidneys, depressed growth, increased water consumption and urine volume, and decreased urine osmolality were noted over the range of dose levels associated with bladder tumors (Schoenig and Anderson, 1985). The similarity of the dose–response curves for neoplastic and nonneoplastic effects strongly suggests an etiological relationship and that a threshold exists below which such effects would not occur. The very narrow dose range over which the dose responses are defined adds to the confidence that can be placed in this interpretation.
EFFECT OF SODIUM SACCHARIN ON BLADDER MORPHOLOGY

The changes that occur in the urothelium of the male rat fed sodium saccharin are critical to an understanding of the mechanism underlying bladder tumor development. Fukushima and Cohen (1980) reported that feeding sodium saccharin at a 5% dietary level to 6-week-old male rats resulted in focal areas of mild simple hyperplasia four to five cells thick in the urothelium at 5 weeks, increasing to five to seven cells thick at 9 weeks. These changes, which were associated with increased numbers of mitotic figures and an increase in thymidine labeling, persisted throughout the 18 weeks of the study. There were no grossly visible changes in the bladder and no calculi were present.

Fukushima and Cohen (1980) also described changes in bladder morphology that occurred using scanning electron microscopy in rats fed sodium saccharin and more recently Cohen et al. (1990) have described the progression in these changes in rats fed 3, 5, or 10% sodium saccharin over a 10-week period. Superficial cells in the process of exfoliation were observed as early as 1 to 3 weeks. After 5 weeks focal denuded areas of the mucosa were observed with exposed underlying basal cells. Cell surfaces in the affected areas were characterized by ropy microridges, and uniform and pleomorphic microvilli, features that are thought to be related to urothelial proliferation. Sodium saccharin did not affect the thymidine labeling index at the 3% dietary level in either of these studies. These findings suggest that in rats fed sodium saccharin superficial urothelial cells become necrotic and are sloughed to initiate the proliferative process that is associated with genetic alteration and tumor development.

The sustained proliferative response present in the rat fed sodium saccharin is analogous to that which occurs in rats with bladder calculi. A variety of substances can give rise to bladder calculi, urothelial proliferation, and bladder tumors in the rat (Clayson, 1974). Urothelial proliferation leading to an increased probability of genetic damage is the common factor in rats with bladder calculi and rats fed sodium saccharin and the basis for the bladder tumor development that occurs in either case.

EPIDEMIOLOGY STUDIES IN HUMANS

The overall conclusion from the extensive epidemiological literature concerning a possible link between saccharin usage and bladder cancer is that saccharin consumption does not increase the risk of bladder cancer in man. Of all the studies conducted to date (approximately 25) only 1 (Howe et al., 1977) reported a significantly increased relative risk for bladder cancer among consumers of products containing saccharin. A more recent study by the same authors (Risch et al., 1988) did not confirm this finding.

Morgan and Wong (1985) have critically analyzed the published epidemiology studies on artificial sweetener use and bladder cancer and have determined the relative risk of bladder cancer when data for cohort, matched pair, and case-control studies were each aggregated. Most of the studies were of the case-control type and of these the authors stated that "on the basis of power analysis, we are 95% certain that, if the true relative risk of bladder cancer as a result of using artificial sweeteners were 1.13 or more, the studies reviewed in this report, in toto, would have detected such a risk as statistically significant." Morgan and Wong (1985) concluded that "saccharin is not related to bladder cancer." Reviews of nonnutritive sweeteners by IARC (1980,
1982) concluded that “the epidemiology data provide no clear evidence that saccharin alone, or in combination with cyclamates, causes urinary bladder cancer” and “there is no consistent evidence that the risk of cancer is increased among users of saccharin.” In a further IARC review of the epidemiological evidence linking saccharin and cyclamates with bladder cancer, Armstrong (1985) stated that with respect to 13 analytical studies “the balance of evidence, based purely on numbers, is against the hypothesis that the use of artificial sweeteners is associated with an increased risk of bladder cancer in humans.” More recently IARC has affirmed that the evidence for carcinogenicity of saccharin to humans is inadequate (IARC, 1987).

OTHER HUMAN STUDIES

The feeding of sodium saccharin over the range of dietary concentrations associated with the development of bladder tumors in the male rat results in physiological changes in the urine and morphological changes in the urothelium. The nature of these changes and the degree to which they have been correlated with tumor development are discussed in previous sections. It was important to determine whether similar changes might occur in humans consuming sodium saccharin. The ingestion of sodium saccharin at a dose of 1 g/day by humans did not affect renal tubular secretion (Sweatman et al., 1981), urine volume (Roberts and Renwick, 1985), or the urinary excretion of bacterial amino acid metabolites (Lawrie and Renwick, 1987). Auerbach and Garfinkel (1989) studied sections from autopsy specimens of bladder from 282 subjects and found no relationship between changes in the urinary bladder and the use of artificial sweeteners.

RISK ASSESSMENT

It is widely accepted that there are two broad types of responses to chemical exposure, each of which requires a different approach to risk characterization. The first of these relates to nonpropagative, often reversible toxic effects that generally occur only at high levels of chemical exposure. The second type of response embraces propagative, self-replicating effects, i.e., tumors, which are not considered to have a no-effect level and for which there is no threshold in the exposure–response relationship. The considerations which aid in differentiating between threshold and nonthreshold responses have been thoroughly reviewed (IRLG, 1979; Truhaut, 1979; Dourson and Stara, 1983; Clayson et al., 1985).

Risk characterization methods differ markedly for these two broad types of responses to chemical exposure. In the first case (i.e., a threshold-type exposure–response relationship), the usual procedure is to estimate a reference dose or an acceptable daily intake for the chemical based on the division of the no-observable-effect level (NOEL) by an appropriate safety factor (e.g., usually 100-fold but larger or smaller depending on the weight of the scientific evidence). NOEL's may be derived from exposures of human populations; however, such data are seldom available and they are generally based on laboratory animal studies.

Risk assessment in the second case (i.e., a nonthreshold-type exposure–response) is based on determination of a risk-specific dose (also known as a virtually safe dose) at some acceptable risk of occurrence of disease (e.g., one in a hundred thousand), using mathematical modeling techniques. A variety of such mathematical models are avail-
able to hypothetically describe the relationship between exposure and effect (Van Ryzin, 1980; Krewski et al., 1982; Carr, 1985; Wilkinson, 1986). These procedures assume mechanisms of toxicity, i.e., genotoxic carcinogenicity, that do not display no-effect levels of exposure.

During recent years advances in knowledge in the field of carcinogenesis suggest that the two-category approach to risk assessment is overly simplistic and, in many cases, scientifically untenable. There are many examples in the literature of substances that produce malignant tumors when administered to experimental animals but for which there are well-defined thresholds and no comparable risk in humans. It is becoming increasingly clear that it is inappropriate to utilize mathematical risk assessment modeling techniques that assume linearity of dose–response and no threshold to the risk assessment determination for all substances that are carcinogenic in laboratory animals. Sodium saccharin is a case in point. As demonstrated in the IRDC study (Schoenig et al., 1985), high levels of exposure to sodium saccharin in the diet of male rats (3 to 7.5% of the diet) resulted in the development of bladder tumors when exposure was commenced early in life and continued throughout the lifetime of the animals. The data from this study clearly demonstrated a nonlinear dose–response relationship with no significant increase in tumor incidence at the 1% level, an equivocal response at the 3% level, and a steep rise between 4 and 7.5% sodium saccharin in the diet. There is a threshold dose for both neoplastic and nonneoplastic effects of sodium saccharin and evidence that these responses are mechanistically interrelated.

Biological Risk Assessment of Saccharin

Biological risk assessment provides an integrated approach that allows consideration of all available scientific evidence in determining the potential human health risks from a particular agent or exposure situation. It has been defined as a qualitative or quantitative approach to the determination of whether an experimentally identified carcinogen will also be effective in human populations and, where possible, whether a carcinogen is more or less active in humans than in laboratory animals (Clayson, 1987). Ethical and practical considerations require that much of the data used in the assessment of potential human health risks be derived from the study of dose–response relationships in nonhuman test systems, including lower animals and various in vitro tests. The interpretation of data derived from these biological test systems is a critical element in the determination of human health risk. High exposure levels to the test materials are often employed in these systems in order to obtain dose–response information. The validity of extrapolating information from high dose animal studies in which mechanisms for handling the test material may be saturated, overloaded, or irrelevant to humans at lower exposure levels must be critically evaluated.

Saccharin in its various forms has been studied more comprehensively than perhaps any other food additive and much is known regarding the mechanism underlying the bladder tumors that occur in male rats fed sodium saccharin. The possibility that sodium saccharin produces bladder tumors in the rat through a classic genotoxic carcinogenic mechanism is remote. Saccharin is not metabolized, it is neither mutagenic nor genotoxic, and it does not react with DNA. Exhaustive studies have also shown that the bladder tumors could not be explained by the presence of mutagenic impurities in saccharin (Herbold, 1981; Riggin et al., 1983) or by the chemical transformation of saccharin under conditions comparable to those in rat urine (Williamson et al., 1987).
Unlike many genotoxic bladder carcinogens, sodium saccharin is a bladder carcinogen only in the rat and almost exclusively in males. Age is also a critical factor in sodium saccharin carcinogenicity. Numerous single-generation studies in the rat wherein sodium saccharin feeding was started after weaning did not result in bladder tumors, whereas positive results were observed when sodium saccharin was fed beginning at birth or at conception (Schoenig et al., 1985).

Urinary physiological changes that are a prerequisite to the development of bladder tumors are a cardinal feature of bladder tumor development in rats fed sodium saccharin or the sodium salts of other organic acids. Numerous studies using bladder tumor promotion models have shown that tumors develop in the rat only with high urinary pH and sodium levels and low osmolality. Tumor promotion does not occur if diets that result in an acidic urine are fed (Imaida and Wang, 1986; Cohen et al., 1991b) nor is bladder tumor promotion observed in rats initiated with FANfT and subsequently fed acid saccharin (Cohen et al., 1991b). Urine saccharin concentrations are comparable in rats fed equivalent dietary levels of acid and sodium saccharin (Hasegawa and Cohen, 1986), suggesting that the saccharin ion is at most a cofactor in bladder tumor development. The bladder tumors that occur when sodium saccharin is fed depend not only on a specific test animal, the male rat, but as well on the presence of specific urine physiological conditions. Tumors do not occur under a variety of experimental regimens in which these conditions are not met or in other species. It has also been shown that conditions which lead to bladder tumor development in the rat fed sodium saccharin also favor the development of a precipitate and silicon-containing crystals in the urine. Cytotoxicity due to silicon crystals and/or precipitate and subsequent hyperplasia is thought to be the ultimate mechanism leading to the formation of bladder tumors.

Although much has yet to be learned about the development of crystalluria in sodium saccharin-fed rats and their effect on the urothelium, an analogy can be drawn between the bladder tumors that occur in rats fed sodium saccharin and develop crystalluria or microcalculi and those found in rats with gross bladder calculi. Bladder calculi associated with tumors occur in rats or mice fed a variety of lithogenic chemicals. Clayson (1974) has emphasized the need for caution in the interpretation of bladder tumors associated with calculi and suggested that they may be the vesicular counterpart of the sarcomas that occur in the subcutaneous tissues of rats and mice implanted with various films and metals (Oppenheimer et al., 1955). Weisburger and Williams (1980) have suggested that “safe” threshold levels of exposure can be established for nongenotoxic “epigenetic” carcinogens whose mechanisms of action are well elucidated. Although this proposal was disputed by Perera (1984) on the grounds that all carcinogens damage DNA, albeit indirectly in some cases. If the formation of crystals or calculi and bladder hyperplasia are required to produce this DNA damage, it is difficult to envisage damage to the genome occurring at urinary concentrations of the agent below those that result in stone or crystal formation. In its Proposed Guidelines for Carcinogen Risk Assessment, the EPA stressed the importance of mechanistic information in determining the relevance of evidence of carcinogenic responses to human health risks (EPA, 1986). The agency apparently followed this dictum in their risk assessment of melamine in which they concluded “the available data on melamine-induced stone formation indicate an operational threshold” (EPA, 1984).

It has been shown that neoplastic and nonneoplastic effects of sodium saccharin in the rat occur over a narrow dose range and only when this sweetener is fed at high dietary concentrations. The similarity of the dose–response curves for both suggests
an etiological relationship and a threshold below which such effects would not occur.
The urothelial damage in the rat that ultimately leads to urothelial proliferation and
tumor development is associated with and dependent on specific urine physiological
changes that would not occur at the level of consumption of this sweetener in humans
which is three to four orders of magnitude less than the doses administered to animals
(NAS, 1978). Furthermore, $\alpha_2\mu$-globulin which has been shown to be a key factor in
the formation of silicate crystals in the rat occurs in only trace amounts in the urine
of man. The protein content of human urine is only 1% of that of male rat urine and
the bulk of the proteins present are high molecular weight rather than the low molecular
weight of rat $\alpha_2\mu$-globulin (Olson et al., 1990). The low protein content of human
urine and the paucity of proteins similar to $\alpha_2\mu$-globulin provide a further biological
basis for suggesting that humans that consume saccharin are not at risk.

CONCLUSIONS

There are few carcinogenic substances that have been more extensively investigated
than sodium saccharin. The evidence which has accumulated supports the conclusion
that the bladder tumors that occur in the male rat and that have been the basis of
concern as to the safety of this sweetener are specific to this species and threshold­
dependent. Furthermore, it has been shown that the mechanism underlying the tumors
is highly unlikely to be operative in humans. These investigative findings are further
supported by the negative results of a large series of human epidemiological studies.
The information on sodium saccharin presently available supports the conclusion that
sodium saccharin does not pose a bladder cancer risk for humans and that application
of a safety factor is an appropriate basis for determining the health risk of this additive.
The NOEL for sodium saccharin in the male rat according to the findings of Schoenberg
et al. (1985) was a dietary level of 1% or approximately 500 mg/kg per day. Applying
a safety factor of 100 yields an acceptable daily intake of 5 mg/kg/day for humans.
The current saccharin exposure level for humans (e.g., fountain soft drinks, drugs,
table top sweeteners, oral hygiene products, chewing gum, etc.) is well below the ac­
ceptable daily intake for this additive.

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