NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for SACCHARIN

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Proposed Report on Carcinogens Delisting for Saccharin[1]

Saccharin is currently listed in the Report on Carcinogens, 8th Edition as *reasonably anticipated to be a human carcinogen.* The basis for this listing was sufficient evidence of carcinogenicity in experimental animals. The Calorie Control Council has petitioned the NTP to consider delisting saccharin from its Report on Carcinogens based upon mechanistic data related to development of urinary bladder cancers in rats.

Carcinogenicity

In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Charles River CD and Sprague-Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations of 1% or greater (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Schoenig et al., 1985). Non-statistically significant increases in urinary bladder cancer have also been seen in saccharin-treated female rats from studies showing a positive effect in males (Arnold et al., 1980; Taylor et al., 1980). Furthermore, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators (e.g., Hicks and Chowaniec, 1977; Cohen et al., 1979; Nakanishi et al., 1980b; West et al., 1986; Fukushima et al., 1990). Several additional rat studies in which sodium saccharin was administered either in the diet or in drinking water were negative for tumorigenicity (Fitzhugh et al., 1951; Lessel, 1971; Schmähl, 1973; cited by IARC, 1980; Chowaniec and Hicks, 1979; Hooson et al., 1980; Schmähl and Habs, 1984).

Three mouse studies have reported positive carcinogenicity following exposure to saccharin. Two of these studies involved surgical implantation of saccharin-containing cholesterol pellets into the urinary bladders and resulted in development of malignant urothelial neoplasms (Allen et al., 1957; Bryan et al., 1970). In the third study, dietary sodium saccharin resulted in increased incidences of malignant thyroid neoplasms (Prasad and Rai, 1986). While the mouse data cannot be discounted, some of these studies had methodological flaws, provided limited information, did not show a dose-response, or had unexpected outcomes that may be species or strain-specific and should be verified by additional studies. Four studies in mice were judged negative for tumorigenesis (Roe et al., 1970; Kroes et al., 1977; Homberger, 1978; Frederick et al., 1989) as were studies in nonhuman primates (McChesney et al., 1977 abstr.; Sieber and Adamson, 1978; both cited by IARC, 1980; Thorgiersson et al., 1994; Cohen et al., 1996 abstr.) and a single hamster study (Althoff et al., 1975).

Much of the epidemiology has examined associations between urinary bladder cancer and artificial sweeteners, rather than saccharin per se. The time trend data for bladder cancer are essentially noninformative with no clear indication that the increased use of saccharin or artificial sweeteners commencing in the 1940s is associated with a general increase in bladder cancer when controlled for confounding factors, chiefly smoking. Risk of bladder cancer in diabetics, who presumably consume greater amounts of artificial sweeteners compared to the general population,

[1]Saccharin is produced commercially as calcium and sodium salts as well as the free acid, and the name saccharin has been applied to all three.

is not greater than risks in the general population (Armstrong and Doll, 1975). Based upon several case-control studies there is no overall association between use of artificial sweeteners and bladder cancer (reviewed by IARC, 1980; IARC, 1987b; JECFA, 1993). It is harder to reject an association between use of artificial sweeteners and bladder cancer in some case-control subgroups, even though the numbers are small[2] (Howe et al., 1980; Hoover and Strasser, 1980; Morrison and Buring, 1980; Cartwright et al., 1981; Morrison et al., 1982; Mommsen et al., 1983). Taken together, while the available epidemiology data show no consistent evidence that saccharin is associated with increased bladder cancer in general, a small increased risk in some subgroups, such as heavy users of artificial sweeteners, cannot be unequivocally excluded. With regard to the general population, if sodium saccharin is a risk factor, it is weak and cannot be proven or disproved due to lack of actual exposure data and intrinsic limitations of existing epidemiology studies.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Extensive studies of the mutagenicity and genotoxicity of saccharin have shown generally negative but occasionally conflicting results. Sodium saccharin is essentially nonmutagenic in conventional bacterial systems but is weakly clastogenic or genotoxic in short-term *in vitro* and *in vivo* test systems (reviewed by Ashby, 1985; IARC, 1987a,b; Whysner and Williams, 1996) with evidence that equimolar ionic solutions of sodium chloride *in vitro* produce a comparable cytotoxic response (Garland et al., 1989a). Urine from mice treated with sodium saccharin was mutagenic in the Ames test (Batzinger et al., 1977). Saccharin does not covalently bind to DNA and does not induce unscheduled DNA synthesis in bladder urothelium.

Saccharin-induced carcinogenesis in rats shows a sex predilection for males (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980), an organ specificity for urinary bladder (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Fukushima et al., 1983; Schoenig et al., 1985), and a dose-response when exposure to dietary concentrations of 1 to 7.5% of the sodium salt of saccharin has begun early in life (beginning at birth or immediately at weaning) and is continued for approximately two years (Schoenig et al., 1985). The results of mechanistic studies have shown that certain physiological conditions must be simultaneously or sequentially present for induction of urinary bladder tumorigenesis. These conditions include a urinary pH greater than 6.5, increased urinary sodium concentration, increased urine volume, decreased urine osmolality, presence of urinary crystals or precipitate, and damage to the urothelium resulting in a proliferative (hyperplastic) response. All of these conditions have been studied extensively in male rats but less so in females. The high levels of urinary protein characteristic of many male rats may partially explain the sex predilection. The high intrinsic rate of urothelial proliferation at about the time of weaning is also believed to contribute to the observed tumorigenic effects. The urinary milieu in rats, especially male rats, is sufficiently different from that in humans or other species to support the contention that these observations are rat-specific. Pharmacokinetic

[2] Morrison and Buring (1980) indicate an increased risk for women. Hoover and Strasser (1980) suggest increased risk among low risk (non-smoking, non-occupationally exposed women) and high risk (male heavy smokers) subgroups.

and metabolism data on sodium saccharin do not explain the male rat sensitivity for induction of urinary bladder neoplasms (Sweatman and Renwick, 1979, 1980).

Conclusion

There is evidence of the carcinogenicity of saccharin in rats but less convincing evidence in mice. Mechanistic studies indicate that the observed urinary bladder cancers in rat studies are related to urinary pH, osmolality, volume, presence of precipitate, and urothelial damage with attendant hyperplasia following dietary concentrations of 3% or higher with inconsistent findings at lower dietary concentrations. The factors thought to contribute to tumor induction by sodium saccharin in rats would not be expected to occur in humans. The mouse data are inconsistent and require verification by additional studies. Results of several epidemiology studies indicate no clear association between saccharin consumption and urinary bladder cancer. Although it is impossible to absolutely conclude that it poses no threat to human health, sodium saccharin is not reasonably anticipated to be a human carcinogen under conditions of general usage as an artificial sweetener.

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Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded; or

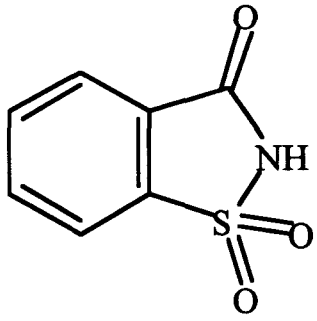
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in previous Reports on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not be reasonably anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Saccharin
[81-07-2]



1.1 Chemical Identification

Saccharin ($C_7H_5NO_3S$, mol. wt. = 183.19) is also called:

Anhydro-*o*-sulfaminebenzoic acid
3-Benzisothiazolinone 1,1-dioxide
1,2-Benzisothiazol-3(2*H*)-one 1,1-dioxide
o-Benzoic sulfimide
Benzoic sulphimide
o-Benzoic sulphimide
o-Benzosulfimide
Benzosulphimide
o-Benzosulphimide
Benzo-2-sulphimide
o-Benzoyl sulfimide
o-Benzoyl sulphimide
1,2-Dihydro-2-ketobenzisosulfonazole
1,2-Dihydro-2-ketobenzisosulphonazole
2,3-Dihydro-3-oxobenzisosulfonazole
2,3-Dihydro-3-oxobenzisosulphonazole
Garantose
Glucid
Gluside
Hermesetas
3-Hydroxybenzisothiazole-*S,S*-dioxide
Insoluble saccharin
Kandiset
Sacarina

Saccharimide
Saccharina
Saccharin acid
Saccharine
Saccharin insoluble
Saccharinol
Saccharinose
Saccharol
Sacharin (Czech)
Sucre edulcor
Sucrette
o-Sulfobenzimide
o-Sulfobenzoic acid imide
2-Sulphobenzoic imide
Zaharina

Saccharin has the RCRA waste number U202.

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	White	HSDB (1996)
Physical State	Monoclinic crystals	Budavari (1996)
Melting Point, °C	228.9-229.7	Budavari (1996)
Density, g/mL	0.828	Budavari (1996)
Odor	Odorless or has a faint aromatic odor	HSDB (1996)
Solubility:		
Water	Soluble in water	Weast and Astle (1980)
Organic Solvents	Soluble in acetone Slightly soluble in chloroform, ethyl ether, and benzene	Weast and Astle (1980); HSDB (1996)
Partition Coefficient:		
Log octanol/water	0.91	HSDB (1996)
Vapor pressure at 25 °C, mm Hg	9.11×10^{-7}	HSDB (1996)

2.0 HUMAN EXPOSURE

Summary: The original uses of saccharin were numerous. Today, it is primarily used as a nonnutritive sweetening agent. From the 1950's to the 1970's, the U.S. consumption of saccharin increased dramatically. Following the ban on saccharin in Canada, stricter legislation on the marketing of saccharin, and the introduction of other artificial sweeteners into the U.S. market, consumption steadily declined. Recently, however, it appears that U.S. saccharin consumption is steady, if not slightly increasing.

Saccharin and sodium saccharin have been produced commercially in the United States for over 80 years. The compounds are produced commercially only by the Maumee process. Calcium saccharin was first produced in the United States in 1953. U.S. imports and production of saccharin has steadily declined. Currently, PMC Specialties Group, Inc. is the only commercial producer of saccharin.

Potential exposure to saccharin occurs through the consumption of dietetic foods and drinks and by use of some personal hygiene products. The concentration of saccharin allowed in these products is regulated by the FDA. Potential exposure to saccharin also occurs in the workplace, specifically in occupations, industries, or facilities that produce and deal with saccharin and its salts.

Regulation of saccharin and its salts is accomplished through many agencies and legislation. The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA). Saccharin is regulated by OSHA under the Hazard Communication Standard.

2.1 Production

The 1979 Toxic Substances Control Act (TSCA) Inventory identified three U.S. companies producing 1.1 million lb (499 metric tons [Mg]) of saccharin in 1977, while 6.3 million lb (2,860 Mg) were imported. Two U.S. companies produced 1.6 million lb (726 Mg) of sodium saccharin, and 281,000 lb (128 Mg) were imported in 1977. Imports of calcium saccharin, which was first produced commercially in the United States in 1953, amounted to 5,500 lb (2.5 Mg) in 1977. One U.S. company produced 550,000 lb (250 Mg) of the ammonium salt in 1977 (NTP, 1994).

Production of all forms of saccharin increased gradually from 180 Mg in 1957 to an estimated 2,040 Mg in 1970 to an estimated total of 2,177 Mg in 1977 (IARC, 1980). The USITC (1981-1991, 1993-1995) identified one U.S. producer of saccharin and its sodium salt from 1980 to 1994, but no production data were provided for these years. The USITC (1983-1985) also reported that one U.S. company produced saccharin, calcium salt, from 1982 to 1984, but no production data were provided. SRI International (1996) identified one U.S. producer of sodium saccharin, most likely PMC Specialties Group, Inc. which produces saccharin under the trade name SYNCAL® in the United States and worldwide (PMC Specialties Group, 1996).

PMC Specialties Group produces sodium saccharin in crystalline and powder forms, and calcium saccharin and insoluble (acid) saccharin in powder form (PMC Specialties Group, 1996). Production volumes were not available.

The forms of saccharin produced by PMC Specialties Group are listed below, in **Table 2-1**.

Table 2-1. Forms of Saccharin Produced by PMC Specialties Group

Trade Name	Synonym	Chemical Formula	CAS No.	Reference
SYNCAL® GS & GSD	soluble saccharin	$(C_6H_4SO_2NCO)Na•2H_2O$	128-44-9	PMC Specialties Group (1997a)
SYNCAL® S & SD	soluble saccharin	$(C_6H_4SO_2NCO)Na$	128-44-9	PMC Specialties Group (1997b)
SYNCAL® CAS	calcium saccharin	$(C_6H_4SO_2NCO)_2Ca$	6485-34-3	PMC Specialties Group (1997c)
SYNCAL® SDI	insoluble (acid) saccharin	$C_7H_5NO_3S$	81-07-2	PMC Specialties Group (1997d)

PMC Specialties Group also produces and markets the SYNCAL® saccharin products SWEET-CHEW® (for animal feed) and SHERBRITE® (for the plating industry) (PMC Specialties Group, 1996).

U.S. imports of saccharin have steadily declined from 5.9 million lb (2,700 Mg) in 1983 to 3.7 million lb (1,700 Mg) in 1984, about 1.8 million lb (817 Mg) in 1985, and 1.6 million lb (726 Mg) in 1987 (NTP, 1994). Calcium saccharin was first produced commercially in the United States in 1953.

Saccharin is manufactured commercially by both the Maumee process and the Remsen-Fahlberg method. In the United States, saccharin and sodium saccharin are produced commercially only by the Maumee process (HSDB, 1996), and have been produced for over 80 years (Crammer and Ikan, 1977; cited by IARC, 1980). In the Maumee process, diazotization of methyl anthranilate by treatment with sodium nitrate and hydrochloric acid gives 2-carbomethoxy-benzenediazonium chloride. Sulfonation of this intermediate gives 2-carbomethyoxy-benzenesulfonic acid, which is treated with chlorine to give 2-carbomethoxy-benzenesulfonyl chloride with chlorine. Treatment of this sulfonyl chloride with ammonia, followed by acidification, gives saccharin (IARC, 1980). Saccharin is converted to the sodium salt by treating with sodium hydroxide or sodium bicarbonate. Twenty-three impurities have been reported in this process (Arnold et al., 1983).

In the Remsen-Fahlberg method of producing saccharin, toluene is reacted with chlorosulfonic acid to produce *o*- and *p*-toluenesulfonyl chlorides. The *o*-isomer is isolated and treated with ammonia to form *o*-toluenesulfonamide. Oxidation gives *o*-sulfamoylbenzoic acid, and when this intermediate is heated, saccharin forms (IARC, 1980). Thirty-one impurities have been reported when saccharin is synthesized by this method (Arnold et al., 1983).

2.2 Use

The primary use of saccharin is as a nonnutritive sweetening agent. Its use increased substantially after cyclamates (synthetic chemicals having a sweet taste) were banned in food in 1969 (FESA database). In 1976, the estimated U.S. consumption for all forms of saccharin was 77% in food uses (45% in soft drinks; 18% in tabletop sweeteners; 14% in fruit juices, sweets, chewing gum, and jellies), and 23% in non-food uses (10% in cosmetics and oral hygiene products, such as toothpastes, mouthwash, and lipstick; 7% in drugs, such as coatings on pills; 2% in smokeless tobacco products, such as chewing tobacco and snuff; 2% in electroplating, e.g., a brightener in nickel-plating baths used in the coating of automobile bumpers; 1% for cattle feed; and 1% in miscellaneous uses (IARC, 1980; HSDB, 1996).

The original uses of saccharin were numerous. A few of the original uses were as an antiseptic and preservative to retard fermentation in food, in estimating the circulation time of blood from an antecubital vein to the lingual capillaries, as an antistatic agent in plastics and textiles, as a polymer modifier and accelerator in photosensitive dispersions, as a light-fastness aid in nylon dyes, and as a chemical intermediate for the fungicide probenazole used in controlling rice blast in Japan (Arnold et al., 1983).

Based upon government legislation and market competition, the consumption of saccharin in the United States has varied. Saccharin and saccharin salts were approved under the 1958 Food Additives Amendment to the Food, Drug, and Cosmetics Act. Under the provisions of this act, saccharin was included in those substances that had been in use prior to 1958 and had been accorded GRAS (Generally Recognized As Safe) status. Saccharin was removed from the GRAS list in 1972, however, when questions by the Food and Drug Administration (FDA) about its safety arose (IARC, 1980). During the period when saccharin was recognized as having GRAS status, its consumption increased dramatically. For example, the consumption of saccharin in the United States in 1953 was 21,000 lb (9.5 Mg); in 1962, 2.5 million lb (1,100 Mg); and following the ban on cyclamates in 1969, consumption rose to 4.0 million lb (1,800 Mg) (Arnold et al., 1983). The approval and introduction of other artificial sweeteners such as aspartame and acesulfame-K into the U. S. market lowered the annual per capita consumer consumption of saccharin from 3.5 kg (9.6 mg/day) in 1980 to 2.7 kg (7.4 mg/day) in both 1985 and 1988 (Irving-Monshaw, 1989). The total U.S. consumption of saccharin in 1992 was 700,000 sugar sweetness equivalent tons (2,333 Mg) whereas aspartame's consumption was 1,500 sugar sweetness equivalent tons (8,333 Mg) (Research Studies-USDA ERS, 1992). According to SRI International, saccharin accounted for 39% of the world's consumption of high-intensity sweeteners in 1992, while aspartame accounted for 41% (Dawson, 1994b). The 1994 consumer consumption of saccharin was estimated to be 2,200 Mg in the United States and 1,100 Mg in Europe (Dawson, 1994a).

In 1983, the Calorie Control Council estimated that in the United States, 44 million adults consumed saccharin-sweetened products (NTP, 1994). It has been estimated that the average consumption of saccharin by humans in the United States is about 5 mg/kg body weight/day (Vesely and Levey, 1978). Saccharin consumption is greatest among diabetics and others whose medical conditions require the restriction of calories or carbohydrates (NTP, 1994).

2.3 Environmental Exposure

2.3.1 Environmental Releases

The Toxic Chemical Release Inventory (EPA) listed four industrial facilities that produced, processed, or otherwise used saccharin in 1988. In compliance with Community Right-to-Know Program, the facilities reported releases of saccharin to the environment which were estimated to total 750 lb (340.5 kg) (NTP, 1994). Facilities are required to notify the National Response Center (NRC) when release of saccharin equals or exceeds its reportable quantity of 100 lb (45.4 kg). When saccharin becomes a waste, as a commercial chemical product, a manufacturing chemical intermediate, an off-specification commercial chemical product, or a manufacturing chemical intermediate, it must be managed according to Federal and/or State hazardous waste regulations (HSDB, 1996).

Releases of saccharin to the environment as reported by PMC Specialties Group, the only U.S. commercial saccharin producer listed by the USITC and Cumberland-Swan, Inc., the manufacturer of Sweet 'n Low®, are listed below, in **Table 2-2.**

Table 2-2. Releases of Saccharin to the Environment

Company	Release	1989	1990	1991
PMC Specialties Group	Air	75 lb/yr (34.1 kg/yr)	65 lb/yr (29.5 kg/yr)	64 lb/yr (29.1 kg/yr)
	Land	0 lb/yr	0 lb/yr	0 lb/yr
	Water	0 lb/yr	0 lb/yr	0 lb/yr
	Sewer	0 lb/yr	10 lb/yr (4.5 kg/yr)	10 lb/yr (4.5 kg/yr)
	Other	1,700 lb/yr (771.8 kg/yr)	1,100 lb/yr (499 kg/yr)	1,400 lb/yr (635.6 kg/yr)
Cumberland-Swan, Inc.	Air		250 lb/yr (113.5 kg/yr)	250 lb/yr (113.5 kg/yr)
	Land		0 kg/yr	0 kg/yr
	Water		0 kg/yr	0 kg/yr
	Sewer		250 lb/yr (113.5 kg/yr)	250 lb/yr (113.5 kg/yr)
	Other		2,700 lb/yr (1,226 kg/yr)	350 lb/yr (158.9 kg/yr)

Source: Toxic Release Inventory Systems (TRIS, 1996)

2.3.2 Environmental Occurrence

Saccharin and its salts, as well as the impurity *o*-toluenesulfonamide, do not occur as natural products (IARC, 1980).

2.3.3 Drinking Water and Food

Refer to section 2.3.4 for any information regarding exposure to saccharin from food.

2.3.4 Consumer Products

Potential exposure to saccharin also occurs through the consumption of dietetic foods and drinks and some personal hygiene products, such as certain toothpastes and mouthwashes that use saccharin as a sweetening agent (NTP, 1994). The FDA has authorized the use of saccharin and its salts in beverages in concentrations not to exceed 12 mg/oz (413 mg/L), as a sugar substitute not to exceed 20 mg for each expressed teaspoonful of sugar sweetening equivalency, and in processed food not to exceed 30 mg per serving. Data from the Nationwide Food Consumption Survey, conducted by the USDA from 1977-1978, on calculated daily saccharin intake levels is presented in **Table 2-3**. The survey included responses from 30,770 U.S. residents from the 48 contiguous states. Respondents reported foods eaten and quantities consumed.

Table 2-3. USDA Nationwide Food Consumption Survey (1977-1978): Total Calculated Saccharin Intake Levels, mg/kg bw/day

Age Group (years); Sex	1-2; M & F	3-5; M & F	6-8; M & F	9-14; M & F	15-18; M	19-34; M	19-34; F	35-64; M
Total Average Daily Intake	11.46	9.62	6.76	5.6	5.23	4.98	5.26	4.96
90th Percentile	15.76	19.67	14.12	11.98	7.4	10.19	10.48	10.48

Source: Calorie Control Council (1996)

The amount of saccharin consumed by diabetics in Great Britain was estimated in a study conducted by researchers at the University of Southampton (MAFF, 1994). The highest level consumed (as measured by the 97.5th percentile) was 3.1 mg saccharin per kilogram body weight per day. The study included 761 participants, age 2 years and over. The average consumption of saccharin by diabetics was not provided.

Consumer exposure to saccharin has possibly decreased in recent years due to the introduction of Nutra-Sweet® (aspartame). According to SRI International, saccharin, packaged as an artificial sweetener under the product name Sweet 'n Low®, commands 31.8% of the U.S. market share in artificial tabletop sweeteners. Saccharin is second to aspartame, which commands 67.8% of the market share (Tomasula, 1994).

2.3.5 Biomarkers of Exposure

Saccharin has not been found to be mutagenic, and evidence shows it does not undergo covalent binding to the DNA of a rat's liver or bladder (Lutz and Schlatter, 1977).

2.3.6 Occupational Exposure

Occupational exposure occurs through dermal contact or inhalation of dust at places where saccharin is produced or used. The risk of potential occupational exposure exists for workers involved in the production of saccharin or its salts, in the manufacture and formulation of saccharin-containing products, and during the packaging of the consumer products. A National Occupational Exposure Survey (1981-1983) estimated that 225,095 total workers, including 97,729 women, representing 73 occupations in 107 industries at 7,347 facilities, potentially were exposed to saccharin (NIOSH, 1990). This survey also found 1,150 employees, including 591 women, representing 5 occupations in 1 industry at 11 facilities were potentially exposed to its sodium salt. This same survey found 10,053 employees, including 4,418 females, representing 16 occupations in 19 industries at 454 facilities that either were involved with the production of, dealt with, or were potentially exposed to sodium saccharin dihydrate (RTECS, 1996).

Table 2-4. NIOSH National Occupational Exposure Survey (NOES, 1981-83)[a]: By Industry

Industry	No. of Plants	No. of Employees	No. of Female Employees
Agricultural Services	230	1838	1608
Heavy Construction Contractors	19	3129	75
Special Trade Contractors	20	1683	
Food and Kindred Products	149	497	
Textile Mill Products	66	252	
Lumber and Wood Products	166	2331	
Furniture and Fixtures	94	2630	376
Paper and Allied Products	132	6134	2295
Printing and Publishing	64	477	
Chemicals and Allied Products	23	1329	175
Rubber and Misc. Plastics Products	52	633	
Stone, Clay, and Glass Products	230	762	
Primary Metal Industries	9	264	
Fabricated Metal Products	307	11616	6172
Machinery, Except Electrical	2107	57361	16608
Electric and Electronic Equipment	881	26850	13490
Transportation Equipment	143	8947	1029
Instruments and Related Products	315	8910	3966
Miscellaneous Manufacturing Industries	86	839	116
Railroad Transportation	22	22	
Trucking and Warehousing	37	75	
Water transportation	39	774	
Transportation by Air	75	10086	57
Communication	152	3748	
Electric, Gas, and Sanitary Services	203	9025	
Business Services	24	1106	24
Auto Repair, Services, and Garages	299	1796	
Miscellaneous Repair Services	704	1110	
Health Services	699	60871	51738
Total	7347	225095	97729

[a]National Institute of Occupational Safety and Health (unpublished provisional data as of July 1, 1990).

2.4 Regulations

2.4.1 Occupational Exposure Limits

No occupational standards or criteria have been promulgated (OSHA) or recommended (NIOSH, ACGIH) in the United States for exposure to saccharin in workroom air.

2.4.2 Other Standards and Criteria

The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). Saccharin is subject to reporting and record keeping rules under CERCLA, RCRA, and SARA. The EPA proposed raising the statutory reportable quantity (RQ) of 1 lb, established under CERCLA, to 100 lb for saccharin and its salts. The final rule adjusts the RQ from 1 lb to 100 lb. Saccharin is regulated as a hazardous constituent of waste under RCRA, and threshold amounts for facilities which may release saccharin have been established under SARA. OSHA regulates saccharin under the Hazard Communication Standard and as a chemical hazard in laboratories. The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA) as a food ingredient not to exceed specific concentrations (NTP, 1994). In compliance with the Delaney Clause, the FDA proposed to ban saccharin as a food additive in 1977 because of the available evidence of its carcinogenicity in animals. Due to conflicting scientific study results as well as the potential benefits of saccharin, a compromise solution was enacted instead of an outright ban. In November, 1977, Congress passed the Saccharin Study and Labeling Act which placed an 18-month moratorium on any action by the FDA against saccharin, and mandated that all products containing saccharin bear the following warning label: "Use of this product may be hazardous to your health. This product contains saccharin, which has been determined to cause cancer in laboratory animals" (Viscusi, 1994). In 1991, the FDA withdrew its call for an outright ban on saccharin in the United States, but warning labels are still required on all packaging (Tomasula, 1994). The moratorium against any further FDA action has been extended to May 1, 1997. FDA regulates, under the Food, Drug, and Cosmetic Act (FD&CA) and the Fair Packaging and Labeling Act, the labeling of various food products containing saccharin and/or saccharin salts. The FDA also regulates how saccharin and certain saccharin salts are used as sweetening agents in food and as a weight control drug under the FD&CA and the Public Health Service Act.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
E P A	40 CFR 261—PART 261—IDENTIFICATION AND LISTING OF HAZARDOUS WASTES. Appendix VII—Basis for Listing Hazardous Waste. Promulgated: 46 FR 4619, 1981 with numerous amendments. The hazardous waste number for saccharin and it salts is U202.	App. VIII lists the hazardous constituents of industrial waste streams listed in 40 CFR 261.31.
	40 CFR 261.30 ff.—Subpart D—Lists of Hazardous Wastes.	
	40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of the substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.
	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Limits: Superfund (CERCLA, SARA) final reportable quantity (RQ) is 100 lb (45.4 kg).	EPA designated as hazardous those substances that when released into the environment may present substantial danger to the public health or welfare or the environment.
	40 CFR 302.6—Sec. 302.6 Notification requirements.	Notification of EPA is required if the RQ is released to the environment.
	40 CFR 372-- PART 372--TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013, 11028. This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986).	Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and aid in the development of regulations, guidelines, and standards.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
E P A	40 CFR 372—Subpart D—Specific Toxic Chemical Listings. 40 CFR 372.65—Sec. 372.65 Chemicals and chemical categories to which this part applies.	
F D A	21 CFR 100—PART 100—GENERAL. Promulgated: 42 FR 14306, 03/15/77. U.S. Code: 21 U.S.C. 321, 331, 337, 342, 343, 348, and 371.	General state and local requirements along with specific administrative rulings and decisions for various food products.
	21 CFR 100.11—Sec. 100.130 Combinations of Nutritive and Nonnutritive Sweeteners in "Diet Beverages".	The label of any "diet beverage" or diet beverage base that contains saccharin must contain the statement "Contains ____ mg saccharin (or saccharin salt, as the case may be) per ounce, a nonnutritive artificial sweetener.
	21 CFR 101—PART 101—FOOD LABELING. Promulgated: 42 FR 14308, 03/15/77. U.S. Code: 15 U.S.C. 1453, 1454, 1455; 21 U.S.C. 321, 331, 342, 343, 348, and 371.	Requirements are given for the principal display panel (the panel most likely to be examined under customary conditions of display for retail sale) of form food.
	21 CFR 101.11—Sec. 101.11 Saccharin and Its Salts; Retail Establishment Notice.	Retail establishments (except restaurants) that sell food containing saccharin shall display a notice informing the consumer that saccharin products are sold at that location.
	21 CFR 150—PART 150—FRUIT BUTTERS, JELLIES, PRESERVES, AND RELATED PRODUCTS. Promulgated: 42 FR 14445, 03/15/77. U.S. Code: 21 U.S.C. 321, 341, 343, 348, 381, and 379e.	Artificially sweetened fruit containing a packing medium sweetened with saccharin and/or sodium saccharin shall have the specified name "artificially sweetened _____", the blank being filled by name of the fruit or fruit product.
	21 CFR 180—PART 180—FOOD ADDITIVES PERMITTED IN FOOD OR IN CONTACT WITH FOOD ON AN INTERIM BASIS PENDING ADDITIONAL STUDY. Promulgated: 61 FR 14482, 04/02/96. U.S. Code: 21 U.S.C. 321, 342, 343, 348, 371; 42 U.S.C. 241.	Regulations govern specific requirements for food additives in food or additives in contact with food. This regulation is pending additional study.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
F D A	21 CFR 180.37—Sec. 180.37 Saccharin, Ammonium Saccharin, Calcium Saccharin, and Sodium Saccharin.	Regulates how these saccharin food additives may be safely used as sweetening agents in food.
	21 CFR 310—PART 310—NEW DRUGS. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	Regulations govern the administrative rulings and decisions on new drug status, new drugs exempted from prescription-dispensing requirements, records, reports, and requests for specific new drugs or devices.
	21 CFR 310.545—Sec. 310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.	There is inadequate data to establish general recognition of the safety and effectiveness of saccharin as a weight control drug product.

The regulations in this table have been updated through the Federal Register 100 Vol.62, May 23, 1997.

3.0 HUMAN STUDIES

A number of epidemiological studies have been conducted to determine whether the use of artificial sweeteners (AS), including saccharin, has been associated with human cancer. U.S. epidemiological studies of AS may not be as informative as those from Canada, the United Kingdom, Europe, and Japan, where widespread saccharin use first began (1945 [imported primarily from Japan and the United States], 1916, 1894, and 1945, respectively). Artificial sweetener use in the United States was not widespread until the middle of the 1960s, when cyclamate and saccharin were used together. The IARC Working Group reviewed saccharin epidemiology in the original monograph (IARC, 1980) and updated the review in Supplement 7 to the IARC Monographs (IARC, 1987b). In both reviews by the IARC Working Group, it was concluded that the results from epidemiological studies of saccharin are equivocal. In a review of saccharin by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993), however, it was concluded that "the epidemiological studies on saccharin did not show any evidence that saccharin ingestion increases the incidence of bladder cancer in human populations".

Epidemiologic studies have in general examined associations between urinary bladder cancer and artificial sweeteners in general, rather than saccharin, per se; this could either inflate or disguise a risk due to saccharin alone. Time trend data are essentially uninformative, since information concerning use of artificial sweeteners and confounding factors is presented only for populations and not for individuals. Cohort studies of diabetics are confounded by reduced smoking in this group. Overall, case-control studies demonstrate at best a small risk for the general population (reviewed in IARC, 1980; IARC, 1987a,b; JECFA, 1993). However, some studies have demonstrated increased risk for groups otherwise at low risk, such as female nonsmokers (Howe et al., 1980; Hoover and Strasser, 1980; Cartwright et al., 1981; Morrison et al, 1982; Mommsen et al., 1983). Heavy users of artificial sweeteners may also be at increased

risk regardless of gender or smoking habits (Hoover and Strasser, 1980). While the available epidemiology data show no consistent evidence that saccharin is associated with increased bladder cancer in general, a small increased risk in some subgroups cannot be excluded.

3.1 IARC (1980) Review of Saccharin Epidemiology

IARC (1980, pp. 171-183; see Appendix A) examined time trends in the United States, England, and Wales and found that there was no marked increase in the incidence of bladder cancer following rapid increase in the use of artificial sweeteners (e.g., see Armstrong and Doll, 1974). In addition, the IARC Working Group found that in the United Kingdom diabetics as a group consume higher quantities of artificial sweeteners and experience lower mortality from bladder cancer than the general population (e.g., see Armstrong and Doll, 1975). The IARC Working Group stated that due to metabolic or dietary differences, use of drugs, exposure to tobacco, or occupational factors associated with diabetics, a carcinogenic effect of sweeteners cannot be excluded (IARC, 1980).

The IARC Working Group evaluated 7 case-control studies (Morgan and Jain, 1974; Simon et al., 1975; Howe et al., 1977; Wynder and Goldsmith, 1977; Miller et al., 1978; Connolly et al, 1978; Kessler and Clark, 1978). Five of the seven studies were negative for bladder cancer and were found to be limited by some inadequacies in experimental design. Of the two studies that examined possible confounding factors in detail, one (Howe et al., 1980 [a reanalysis of data from Howe et al., 1977]) suggested that artificial sweetener use was positively associated with bladder cancer in men but not in women. The association was limited to men who consumed an average of more than eight tablets of saccharin per day or men who used nine or more tablets of AS per day. In both instances, the relative risk (RR) was approximately 3. The IARC Working Group noted that in these small groups, the result could have been due to confounding factors that were not included in the analysis, residual confounding effects of those factors that were considered in the analysis, or chance.

The second study reviewed by the IARC Working Group that considered confounding factors (Kessler and Clark, 1978; cited by IARC, 1980) found no association between bladder cancer and use of AS and suggested that a relative risk of about 1.5 or higher was unlikely.

In 6 out of 7 of the case-control studies reviewed by the IARC Working Group, women with bladder cancer consumed less AS than the controls. The IARC Working Group stated that this observation suggests that there is no association between use of artificial sweeteners and bladder cancer in women.

In a case-control study that was in press when reviewed by IARC (1980), Wynder and Stellman (1980) reported that there was no association between use of artificial sweeteners or diet beverages and bladder cancer. The study included 302 male and 65 female bladder cancer patients who were matched by age, sex, hospital, and hospital-room status to an equal number of patients without bladder cancer. More details on this study after publication are given in subsection 3.2.1.

The 1980 IARC Working Group concluded their review of epidemiological data for AS with the following statement: The epidemiological data taken as a whole cannot with confidence exclude a small increase in risk but provide no clear evidence that artificial sweeteners cause bladder cancer in humans (IARC, 1980). In 1987, the IARC Working Group reiterated the

findings from their 1980 review by concluding that the evidence that the risk of cancer is increased among users of artificial sweeteners is inconsistent (IARC, 1987).

3.2 Human Studies Published Post IARC (1980)

Experimental details for the studies described in this section are presented in **Table 3-1**.

3.2.1 U.S. Case-Control Studies

Hoover and Strasser (1980) conducted a large multicenter bladder cancer case-control study that included 3010 newly diagnosed, histologically confirmed bladder cancer cases and 5783 population-based controls chosen at random. Information collected by personal interview included information regarding quantity of AS consumed, either by table-top or diet-drink use. No increase in overall RR for bladder tumors was found when comparing the use of AS with never having used AS (males: RR = 0.99; CI [Confidence Interval] = 0.89-1.10; females: RR = 1.07; CI = 0.89-1.29). There was no trend found for men for either table-top or diet-drink AS use. A statistically significant trend for table-top, but not diet-drink, consumption was observed for females after adjustment for age, race, and cigarette smoking. For men and women who consumed at least 2 diet drinks and 3 table-top servings/day or at least some diet drinks and at least 6 table-top servings/day, there was a borderline statistically significant RR of 1.45 (CI = 1.00-2.10) after adjustment for sex, age, race, smoking, occupational exposures, region, and education (for males the RR was 1.47; for females the RR was 1.41). Two subgroups—females who had never smoked or been occupationally exposed to known bladder carcinogens and men who smoked heavily—showed a statistically significant relative risk estimate with daily AS use (men: table-top $\geq$6 uses, RR = 1.86; diet drinks $\geq$3 servings, RR = 2.62; women: table-top $\geq$2 uses for $\geq$5-9 years, RR = 1.8; $\geq$2 uses >10 years RR = 2.7). Additional control for coffee drinking, history of geographic area, education, obesity, use of hair dyes, and history of urinary infections did not affect the relative risk. [IARC (1982) reviewed this study in Supplement 4 to the monographs.]

Using a different analytic approach, Walker et al. (1982) reevaluated the study conducted by Hoover and Strasser (1980) and found essentially the same overall result for AS use (RR = 1.2; CI = 1.0-1.5). These investigators used a composite variable that included education, bladder infection, job exposure, and coffee consumption to define baseline risk strata. Odds ratio estimates were adjusted for region, race, sex, and age. The authors found no trends in odds ratios associated with increasing AS use for the different risk categories. However, this reanalysis was criticized by Hoover and Hartge (1982; cited by IARC, 1987b), who argued that the use of stratification did not include sex and age, and suggested that the low- or high-risk groups based on the composite risk variable used in the reanalysis were actually of intermediate risk. [IARC (1982) mentioned these two studies in Supplement 4.]

Morrison and Buring (1980) reported an association of artificial sweetener use and increased risk of lower urinary tract cancer in females. The relative risk of lower urinary tract cancer was 1.6 (95% CI = 0.9-2.7; 69 cases/46 controls) among women who never used dietetic beverages, and 1.5 (95% CI = 0.9-2.6; 54 cases/39 controls) among women who reported use of sugar substitutes. There was also an increased lower urinary tract cancer risk among women after five or more years of dietetic beverage use (RR = 3.7; 22 cases/6 controls), but statistical

estimates were not provided. [This study was described by IARC (1980) as a footnote since it was published after the Working Group Meeting.]

A case-control study was conducted by Wynder and Stellman (1980) between 1977 and 1979 using 302 male and 65 female cases with bladder cancer. Controls were hospital admissions matched for sex, age, hospital, and hospital-room status (an indicator of socioeconomic status). The authors found no association between use of saccharin or diet beverages and bladder cancer. The RR for saccharin use was 0.93 (CI = 0.68-1.28) for men and 0.62 (CI = 0.26-1.40) for women. For diet beverage consumption, the RR was 0.85 (CI = 0.55-1.17) for men and 0.60 (CI = 0.27-1.29) for women. [This study was published after the Working Group meeting and was described in IARC (1980) as a footnote.]

Najem et al. (1982) compared 75 male and female bladder cancer cases with 142 hospital-based controls in a study conducted in 1978 in New Jersey. Controls were matched to cases by age, place of birth, sex, race, source of obtaining cases, and place of current residence. The authors found no statistically significant increased risk of bladder cancer from consumption of saccharin (RR = 1.3 [CI = 0.6-2.8]). However, only 12/75 cases (16%) and 19/142 controls (13%), reported having consumed saccharin. The relative risk was not adjusted for any potentially confounding factors.

Silverman et al. (1983) examined the use of population- versus hospital-based controls to estimate the risk of lower urinary tract cancer from AS consumption. The study was conducted in Detroit, MI as an add-on to the multicenter study conducted by Hoover and Strasser (1980). The study included 391 cases diagnosed from December 1977 to December 1978 in Detroit with transitional or squamous cell carcinoma of lower urinary tract, 305 population-based controls matched to cases by age and sex, and 440 hospital-based controls discharged from the same hospital as a case and matched by age, race, sex, discharge date. Population-based controls had a lower reported AS use compared with hospital-based controls. Using population-based controls, the RRs for men and women were 1.1 and 1.8, respectively. Using hospital-based controls, the RRs for men and women were 0.9 and 1.1, respectively. Using hospital controls without obesity-related diseases, RR was 1.1 for both men and women. Adjustment of RR values for age, smoking, education, and body mass index were found to have no effect on risk.

A New York state study reported no increased risk of bladder cancer for young (20 to 49 yr-old) women who reported using AS more than 100 times (Odds Ratio [OR] = 1.1 [CI = 0.7-1.7]). Cases (173) with bladder cancer diagnosed between 1975 and 1980 were matched by sex, age, and residence within an area code to 173 population-based controls (Piper et al., 1986).

In a study conducted by Nomura et al. (1991), men and women of Japanese or Caucasian ancestry, diagnosed with lower urinary tract cancer between 1977 and 1986 in Oahu were matched to population-based controls by sex, ethnic group, age, and residence. Participants were classified into non-users and users of saccharin based on consumption history 1 year prior to interview or diagnosis. There was no increased risk of lower urinary tract cancer in users (OR for men, 1.1 [CI = 0.7-1.8]; OR for women, 0.7 [CI = 0.3-1.5]).

In an analysis of data from the Hoover and Strasser (1980) study conducted by Sturgeon et al. (1994), it was found that heavy use of AS (≥ 1680 mg/day) was associated with higher-grade, poorly differentiated bladder tumors (RR = 2.2; CI = 1.3-3.6). The analysis included 1860 cases from 10 geographic regions with bladder cancer identified between December 1977 and

March 1978, and 3934 population-based controls. The RR was adjusted for age, sex, cigarette use, history of urinary infection or bladder stones, coffee consumption, family history of urinary tract cancer, high-risk occupation, race, and education.

3.2.2 Canadian Case-Control Studies

Risch et al. (1988) conducted a large multicenter Canadian bladder cancer study that matched 826 cases with population-based controls during 1979-1982. No association with any table-top AS consumption, including a subgroup of nonsmoking females (OR = 1.04; CI = 0.4-2.71) was reported. An OR of approximately 2 was associated with females that drank diet soda; the dose-related trend reached borderline statistical significance. The authors noted that none of the diet soda consumption had exceeded 10 years (Risch et al., 1988). Thus, these authors failed to confirm the increased risk for bladder cancer that they previously reported (Howe et al., 1980) for consumers of artificial sweeteners.

3.2.3 Case-Control Studies From Other Countries

Morrison et al. (1982) conducted a case control study including cases of lower urinary tract cancer cases from Nagoya, Japan (293 cases) and Manchester, United Kingdom (555 cases). Controls (589 Japanese, 735 British) were population-based and were matched to cases by age and sex. The study found no increased risk of lower urinary tract cancer related to AS use (British men, RR = 0.9 [CI = 0.7-1.2]; British women, RR = 0.9 [CI = 0.6-1.4]; Japanese men, RR = 0.7 [CI = 0.5-0.9]; Japanese women, RR = 0.5 [CI = 0.3-0.8]). The study populations from Japan and the United Kingdom used saccharin predominantly (97% of British, 94% of Japanese) for 30-40 years prior to the study. The authors found an increased RR of 1.6 among nonsmoking men from the United Kingdom; the RR for nonsmoking British women was 1.2. There was no increased risk in nonsmoking Japanese or in any group of current or former smokers. The United Kingdom analysis for AS in tablets showed an increased RR among the over-10-tablets-a-day female group (RR = 2.3) and a decrease in males (RR = 0.6).

Another study from the United Kingdom, conducted by Cartwright et al. (1981), included 622 prevalent and 219 incident cases of bladder cancer in West Yorkshire, each of which was matched to hospital-based controls (622 for existing cases, 448 for new cases) for age and sex. Saccharin use was described as regular for > 1 year, at least 5 years prior to diagnosis. Risk was significantly elevated for nonsmoking males (RR = 2.2 [CI = 1.3-3.8]), but not for nonsmoking females (RR = 1.6 [CI = 0.8-3.2]), or for smokers of either sex (male RR = 0.9 [CI = 0.6-1.3]; female RR = 1.2 [CI = 0.5-2.6]). The RR values were adjusted for age and type of case (incident or prevalent).

Mommsen et al. (1983) conducted a small case-control study from Denmark comprised of 47 female cases newly diagnosed with bladder cancer and 94 population-based controls matched by sex, age, and geographic area, including degree of urbanization. Cases were interviewed in person at the hospital, whereas controls received a mailed questionnaire which was followed up by a phone interview. Only 6/47 cases and 2/94 controls reported consumption of saccharin. An elevated risk of bladder cancer was found for all women who had consumed saccharin (RR = 6.7 [CI = 1.5-30.2]). When only nonsmokers who used saccharin were included, the risk decreased (RR = 3.3 [CI = 1.4-7.8]).

In another study from Denmark, however, Møller-Jensen et al. (1983) found no increased risk of bladder cancer from consumption of saccharin (RR for men = 0.68 [CI = 0.45-1.02]; RR for women = 1.04 [CI = 0.51-2.09]). The study included 290 male and 98 female bladder cancer patients who were matched by age and sex to 592 male and 195 female controls selected at random from the general population. Participants were classified as users of saccharin only (72.9%), cyclamate only (10.7%), or both substances.

A case-control study using 117 cases with 117 population-based controls and 117 hospital-based controls was prompted following a report of high bladder cancer incidence in La Plata, Argentina. However, no association between saccharin use and bladder cancer was reported. Controls were matched to cases by sex, age, and residence (population-based controls) or hospital (hospital-based controls). Relative risk values were not provided (Iscovich et al., 1987).

No increased risk of bladder cancer from consumption of saccharin (as a food additive only) was found in a case-control study conducted by Momas et al. (1994) (OR = 1.5 [CI = 0.8-3.0]). The study included 219 men living in a region of France for > 5 years and diagnosed with primary bladder carcinoma between January 1987 and May 1989. The 794 controls were men from the same region who were over 50 years old and had lived in the region > 5 years. Saccharin use was defined as consumption of 365 (units were not given).

3.2.4 Descriptive Studies

Jensen and Kamby (1982) found that *in utero* exposure to saccharin did not appear to increase the bladder cancer incidence in the first 3 decades of life, which was the limitation of their follow-up. This Danish study also found no increased incidence in bladder cancer mortality up to an age of 30 years for persons born from 1941 to 1945, which corresponds to a time period when saccharin use was high in Denmark due to war-time shortages of sugar.

3.2.5 Meta-Analysis

In a meta-analysis that included 12 case-control studies on the relationship between AS and bladder cancer incidence, Elcock and Morgan (1993) estimated a summary RR of near unity (males, 0.958; females; 0.961).

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control	cases: men and women from diagnosed with carcinoma of urinary bladder in 10 geographic regions between Dec. 1977 and Dec. 1978; aged 21-84 yr (cases with history of urinary tract cancer were excluded) controls: age and sex stratified random sample of the general population from the same 10 geographic regions	cases: 3010 controls: 5783 75% of cases and controls were males	cases: 87 controls: 85 (aged 21-64 yr); 87 (aged 65-84)	artificial sweetener	lifetime	never used artificial sweetener ever used diet drink ever used tabletop artificial sweetener ever used diet food ever used any form	Relative Risks: M: 1.00 F: 1.00 M: 0.95 (0.84-1.07) F: 1.02 (0.83-1.25) M: 1.04 (0.92-1.18) F: 1.04 (0.84-1.28) M: 1.02 (0.85-1.22) F: 1.13 (0.87-1.47) M: 0.99 (0.89-1.10) F: 1.07 (0.89-1.29)	race, cigarette use, coffee consumption, occupational exposure (additional control for age, sex, history of diabetes, geographic area, and education did not affect RR)	yes, for two subgroups (non-smoking females; heavy smoking males)	personal interview in home of participants	Hoover and Strasser (1980)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)							Relative Risks for Use of Artificial Sweeteners in Subgroups: no. cases/controls:				Hoover and Strasser (1980)
	Low-Risk White Females (never smoked, no occupational exposures)	cases: 130 controls: 402		table-top sweeteners table-top sweeteners table-top sweeteners	5 years 5-9 years > 10 years	≥ 2 uses per day ≥ 2 uses per day ≥ 2 uses per day	1.3; 14/34 1.8; 13/22 2.7; 16/18 all RR had p < 0.01; 95% CI not provided	age			
	High-Risk White Males (smoked more than 40 cigarettes per day)	cases: 104 controls: 167		table-top sweeteners table-top sweeteners table-top sweeteners table-top sweeteners table-top sweeteners diet drinks diet drinks diet drinks		< 1 uses per day 1-1.9 uses per day 2-3.9 uses per day 4-5.9 uses per day ≥ 6 uses per day 1-1.9 servings per day 2-2.9 servings per day ≥ 3 servings per day	1.28; 12/15 2.07; 19/14 1.96; 16/13 1.33; 8/10 1.86; 7/7 1.20; 14/19 3.33; 10/5 2.62; 6/4 all RR had p = 0.01; 95% CI not provided	age			Hoover and Strasser (1980)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases admitted to Boston hospitals for first primary neoplasm of the lower urinary tract from March 1976 through May 1977; controls from general population of study area	592 cases, 94% with bladder tumors and 74% male; 536 controls	cases: 81% controls: 80%	artificial sweetener	years of use: <5, 5-9, more than 10	no. drinks per day; no. sugar substitutes per day; no. dietetic food servings per week	Relative Risks for Lower Urinary Tract Cancer and Ever-Use of Artificial Sweeteners: no. case/controls M and F: 0.9 (0.7-1.2) for dietetic beverages or sugar substitutes **Dietetic Beverage Use History** M: 0.8 (0.6-1.1); 144/155 F: 1.6 (0.9-2.7); 69/46 **Sugar Substitute Use History** M: 0.8 (0.5-1.1); 101/113 F: 1.5 (0.9-2.6); 54/39	age, sex, smoking history	weak because of low numbers of cases and controls and no statistical estimates of confidence for duration of use	interviews with subjects or proxies if subjects too ill, could not be contacted, or deceased	Morrison and Buring (1980)
				diet drinks	< 5 yr		M: 1.1; 62/59				
				diet drinks	> 10 yr		M: 0.7; 17/21				
				diet drinks	< 5 yr		F: 1.0; 27/27				
				diet drinks	≥ 5 yr		F: 3.7; 22/6				
							CI and p value not provided				

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: men and women with a first diagnosis of bladder cancer and admitted to hospital; interviewed between Aug. 1977 and June 1979 controls: patients admitted to hospital for other neoplastic and nonneoplastic conditions; matched to cases by age, sex, hospital, and hospital-room status	cases: 367 controls: 367	not specified	saccharin	≥ 10 yr	≥ 40 mg saccharin/day (as artificial sweetener) ≥ 2 cans diet beverage/day (≥192-264 mg saccharin/day)	Relative Risk: M: 0.93 (0.68-1.28) F: 0.62 (0.26-1.40) M: 0.85 (0.55-1.17) F: 0.60 (0.27-1.29)	RR did not vary when adjusted for history of diabetes, obesity, occupation, education, religion, coffee or tea consumption, and cigarette use (data not provided)	no	personal interview in hospital	Wynder and Stellman (1980)
	cases: men and women with bladder cancer, but with no tobacco-related heart disease, admitted to hospitals/clinics in New Jersey during 1978; mean age, 66.8 yr controls: admitted to hospitals/clinics for other conditions, excluding tobacco-related heart disease and any neoplasm; matched to cases by age, place of birth, sex, race, source of obtaining cases, current residence; mean age, 70.9 yr	cases: 75 controls: 142	not specified	saccharin	not specified	regularly consumed vs. never or occasionally consumed	Risk Ratio: 1.3 (0.6-2.8) not significant; p > 0.05 (cases consumed an average of 3.6 tablets/day for a mean period of 6.4 yr; controls consumed an average of 2.5 tablets for 6.3 yr)	none	no	all cases and controls interviewed by 1 nurse; responses recorded on precoded form	Najem et al. (1982)
	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	Relative Risk: 1.2 (1.0-1.5)	age, sex, race, religion	no	re-evaluation of Hoover & Strasser (1980) data	Walker et al. (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: diagnosed from December 1977-December 1978 in Detroit with transitional or squamous cell carcinoma of lower urinary tract; aged 21-84 yr hospital-based controls: residents of Detroit, discharged from same hospital as case; matched by age, race, sex, discharge date population-based controls: matched by age and sex	cases: 391 hospital-based controls: 305 population-based controls: 440	cases: 91 hospital-based controls: 89 population-based controls: 91	artificial sweetener	lifetime	ever or never used	Relative Risk: using population controls: 1.1 (men); 1.8 (women) using hospital controls: 0.9 (men); 1.1 (women) using hospital controls without obesity-related diseases: 1.1 (men); 1.1 (women)	none (adjustment for age, smoking, education, and body mass index had no effect on relative risk)	not applicable	questionnaire given in person, by phone (only if necessary), or by proxy (only if necessary); add-on to Hoover and Strasser (1980) study	Silverman et al. (1983)
	cases: men and women of Japanese or Caucasian ancestry, diagnosed with lower urinary tract cancer between 1977 and 1986 in Oahu; aged 30-93 yr controls: population-based; matched to cases by sex, ethnic group, age, and residence	cases: 261 controls: 522	cases: 86 controls: 89	saccharin	1 yr	non-user user 1-5 serving-yr 6+ serving-yr	Odds Ratio: M: 1.0; F: 1.0 M: 1.1(0.7-1.8) F: 0.7(0.3-1.5) M: 1.2(0.6-2.4) F: 0.5(0.2-1.6) M: 1.1(0.6-1.9) F: 0.9(0.3-2.9)	cigarette use	no	personal interview in home of participant	Nomura et al. (1991)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: women diagnosed with bladder cancer in New York state between January 1975 and September 1980; aged 20-49 controls: population-based; matched to cases by sex, age, and residence within an area code	cases: 173 controls: 173	cases: 80.8 controls: 71	artificial sweetener	ever used artificial sweetener ≥ 100 times	not specified	Odds Ratio: 1.1 (0.7-1.7)	none	not specified	telephone interview during 1982	Piper et al. (1986)
	cases: men and women diagnosed with transitional cell bladder cancer between 1977 and 1978 in 10 geographic regions; aged 21-84 years controls: randomly selected from general population	cases: 1860 controls: 3934	cases: 73 controls: 83	artificial sweetener	lifetime	< 1680 mg/day ≥ 1680 mg/day	Relative Risk: noninvasive: 1.0 invasive: 1.0 Grade I: 1.0 Grade II: 1.0 Grade III/IV: 1.0 noninvasive: 1.3 (0.9-2.1) invasive: 1.3 (0.8-2.3) Grade I: 1.1 (0.5-2.3) Grade II: 1.1 (0.6-2.0) Grade III/IV: 2.2 (1.3-3.6)	age, sex, cigarette use, history of urinary infection or bladder stones, coffee consumption, family history of urinary tract cancer, high-risk occupation, race, education	not specified	personal interview in home of participant	Sturgeon et al. (1994)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Canada Case-control	cases: men and women newly diagnosed with urinary bladder cancer between 1979 and 1982 in Alberta or southcentral Ontario; aged 35-79 yr controls: randomly selected, population-based; matched to cases by age, sex, and area of residence	cases: 826 controls: 792	cases: 67 controls: 53	saccharin	lifetime	30 usage-yr (Usage-years represent cumulative exposure, e.g., 3 uses/ day for 10 yr=30 usage yr)	Odds Ratio: M: 1.01 (0.86-1.18) F: 0.96 (0.79-1.16)	lifetime cigarette consumption and history of diabetes	no	interview in home of participant	Risch et al. (1988)
Other Case-control	cases: residents of Manchester, United Kingdom or Nagoya, Japan diagnosed in 1976-1978 with lower urinary tract cancer; aged 21-89 controls: population-based, matched to cases by age and sex	cases: 555 British, 293 Japanese controls: 735 British, 589 Japanese	cases: 96 (British), 84 (Japanese) controls: 90 (British), 80 (Japanese)	artificial sweetener (97% of British and 94% of Japanese used saccharin)	~ 30-40 yr (most reported first use during or shortly after start of World War II)	ever or never used	Relative Risk: British men: 0.9 (0.7-1.2) British women: 0.9 (0.6-1.4) Japanese men: 0.7 (0.5-0.9) Japanese women: 0.5 (0.3-0.8) There was an increased RR of 1.6 among nonsmoking men from the United Kingdom. RR was not increased for any other nonsmoking group or for any current or former smokers group.	stratified by age (< 65 yr, 65-74 yr, or 75+ yr) Preliminary analysis of British men revealed no effect of occupational history on risk	British cases had an increased RR among the over-10-tablets/day female group (RR = 2.3), but not for 10-tablets/day male group (RR = 0.6). Japanese not evaluated for dose-response. There was no association between duration of use and increase in risk for British or Japanese.	interview in home (British cases/ controls, Japanese controls) or interview in hospital (Japanese cases)	Morrison et al. (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Other Case-control (cont.)	cases: men and women newly and previously diagnosed with bladder cancer in West Yorkshire, United Kingdom controls: hospital-based; matched to cases by age and sex	cases: 622 existing cases, 219 new cases controls: 622 for existing cases, 448 for new cases	not specified	saccharin (as food additive only)	> 1 yr, beginning at least 5 yr before cancer diagnosis	user or non-user	Relative Risk: male nonsmokers: 2.2 (1.3-3.8) female nonsmokers: 1.6 (0.8-3.2) male smokers: 0.9 (0.6-1.3) female smokers: 1.2 (0.5-2.6)	age and type of case (new or existing)	not specified	personal interview (site not specified); cases and controls interviewed by same person	Cartwright et al. (1981)
	cases: women from Denmark newly diagnosed with bladder cancer, average age, 66.4 yr controls: population-based; matched to cases by sex, age, and geographic area, including degree of urbanization	cases: 47 (of the 47 cases, only 6 had consumed saccharin) controls: 94 (of the 94 controls, only 2 had consumed saccharin)	cases: 81 controls: 100	saccharin	not specified	not specified	Relative Risk: all women: 6.7 (1.5-30.2) never-smokers only: 3.3 (1.4-7.8)	none	not specified	cases: personal interview in hospital controls: mailed questionnaire followed by phone interview	Mommsen et al. (1983)
	cases: men and women diagnosed with bladder cancer in Copenhagen, Denmark between May 1979 and April 1981 controls: residents of Copenhagen, randomly selected; matched to cases by age and sex	cases: 388 controls: 787	cases: 94.4 controls: 75.1	artificial sweetener (72.9% used saccharin alone; 10.7% used cyclamate alone; 16.4% used both)	≥ 3 mo	never used or ever used	Relative Risk for Users of Saccharin Alone: M: 0.68 (0.45-1.02) F: 1.04 (0.51-2.09)	not specified	no	interview in home of participant	Møller-Jensen et al. (1983)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Other Case-control (cont.)	cases: men and women living in La Plata, Argentina for ≥ 5 yr and diagnosed with bladder cancer population-based controls: matched to cases by sex, age, residence (street block) hospital-based controls: matched to cases by sex, age, hospital	cases: 117 population-based controls: 117 hospital-based controls: 117	not specified	saccharin	not specified	not specified	RR not specified, but labeled as not significant	not specified	no	personal interview in home (population controls) or hospital (cases and hospital controls)	Iscovich et al. (1987)
	cases: men living in the Hérault region of France for ≥ 5 yr and diagnosed with primary bladder carcinoma between Jan. 1987 and May 1989 controls: randomly selected men from Hérault region; only men over 50 yr old who had lived in Hérault region > 5 yr were included	cases: 219 controls: 794	cases: 80.5 controls: 77.8	saccharin (as a food additive only)	lifetime	< 365 or ≥ 365 (units not specified)	Odds Ratio: 1.5 (0.8-3.0)	not specified	not specified	telephone interview or mailed questionnaire (for those not listed in phone book)	Momas et al. (1994)
Descrip-tive	cohorts: residents of Denmark born between 1941 and 1945 (when saccharin use was high); evaluated from 1961-1976 (aged ≤ 34 yr) controls: residents of Denmark born 1931-1940	not specified	not specified	saccharin	≤ 30 yr (exposure beginning *in utero*)	not specified	There was no increase in bladder cancer mortality during the first 3 decades of life in cohorts.	not specified	no	observed cases in cohorts compared to expected cases (i.e., cases among those born 1931-1940)	Jensen and Kamby (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Meta-analysis	multiple sources	cases: 5499 M, 2082 F controls: not specified	not specified	artificial sweetener	not specified	not specified	Relative Risk: M[b]: 0.958 (0.69-1.33) F[b]: 0.961(0.85-1.08) all studies[c]: 0.979 (0.92-1.04)	RR inversely weighted by the variance from each study	not specified	meta-analysis of 13 case-control studies	Elcock and Morgan (1993)

Abbreviations: F = female; M = male; OR = odds ration; RR = relative risk

[a] Unless otherwise noted, the type of artificial sweetener consumed was not specified
[b] This category included 12 studies
[c] This category included 13 studies

4.0 MAMMALIAN CARCINOGENICITY

Several conventional carcinogenicity studies of dietary sodium saccharin have been conducted in rats. Four of these studies that meet contemporary standards for hazard identification, including absence of urinary bladder parasites, have shown induction of neoplasia in urinary bladder urothelium of male rats. A condition that appears to be necessary for positive results is exposure to high doses of sodium saccharin close to the time of weaning with continued exposure for two years. In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Charles River CD and Sprague-Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations of 1% or greater (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Schoenig et al., 1985) and statistically significant increased bladder neoplasia at 4% or greater (Schoenig et al., 1985; Squire, 1985). Non-statistically significant increases in urinary bladder cancer have also been seen in saccharin-treated female rats from studies showing a positive effect in males (Arnold et al., 1980; Taylor et al., 1980). Furthermore, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators (e.g., Hicks and Chowaniec, 1977; Cohen et al., 1979; Nakanishi et al., 1980b; West et al., 1986; Fukushima et al., 1990). Several additional rat studies in which sodium saccharin was administered either in the diet or in drinking water were negative for tumorigenicity (Fitzhugh et al., 1951; Lessel, 1971; Schmähl, 1973; Chowaniec and Hicks, 1979; Hooson et al., 1980; Schmähl and Habs, 1984).

Conventional carcinogenicity studies of dietary sodium saccharin in mice have been less rigorously carried out, and have been negative for urinary bladder carcinogenesis. On the other hand, two studies in which saccharin-containing cholesterol pellets were surgically implanted into the urinary bladders of mice have yielded urinary bladder cancers. Three mouse studies have reported positive carcinogenicity following exposure to saccharin. Two of these studies involved surgical implantation of saccharin-containing cholesterol pellets into the urinary bladders and resulted in development of malignant urothelial neoplasms (Allen et al., 1957; Bryan et al., 1970). In the third study, dietary sodium saccharin resulted in increased incidences of malignant thyroid neoplasms (Prasad and Rai, 1986). While the mouse data cannot be discounted, some of these studies had methodological flaws, provided limited information, did not show a dose-response, or had unexpected outcomes that may be species or strain-specific and should be verified by additional studies. Four studies in mice were judged negative for tumorigenesis (Roe et al., 1970; Kroes et al., 1977; Homberger, 1978; Frederick et al., 1989) as were studies in nonhuman primates (McChesney et al., 1977 abstr.; Sieber and Adamson, 1978; both cited by IARC, 1980; Thorgiersson et al., 1994; Cohen et al., 1996 abstr.) and a single hamster study (Althoff et al., 1975).

4.1 Mammalian Carcinogenicity of Saccharin

Full experimental details for the studies described in this section are presented in **Table 4-1**.

4.1.1 Hamsters

No urinary tract tumors were observed in Syrian golden hamsters exposed to 0.156-1.25% sodium saccharin in drinking water for life (50-60 weeks). The incidence of tumors in other tissues was within the range of spontaneously occurring tumors (Althoff et al., 1975).

4.1.2 Mice

Twenty-five days after application of saccharin to the skin (8% solution in acetone), "S" strain mice were given 18 weekly applications of 0.17% croton oil in acetone. Following treatment with croton oil, 14 skin tumors were observed in 7/20 mice exposed to saccharin, while 4 skin tumors were observed in 4/19 control mice treated with croton oil only. The difference was not significant (p value not given) (Salaman and Roe, 1956; cited by IARC, 1980).

An increased incidence of bladder cancer ($p = 0.01$; χ^2 test) was observed in "stock" mice that had saccharin/cholesterol pellets (2 mg saccharin/8 mg cholesterol) implanted in their urinary bladder lumina for 40 or 52 weeks (Allen et al., 1957). The authors noted that the presence of the cholesterol pellet in the bladder may have had a promoting action, and that the method of bladder implantation detects incomplete carcinogens. It was not specified whether other tissues were examined. The saccharin used was of unknown purity and the study involved small numbers of animals whose sex was not specified.

As part of a combined carcinogenesis and tumor promotion study (Roe et al., 1970), female Swiss mice were given a 5% saccharin diet for 18 months. Based upon macroscopic examination of all major organs except brain, pituitary, and spinal cord, there were no alterations in gross lesions or tumor incidences in saccharin-treated mice. The necropsy included careful macroscopic examination of urinary bladder.

Stoner et al. (1973; cited by IARC, 1980) found that intraperitoneal (i.p.) saccharin exposure (8 weeks, 0.6 or 3.3 g/kg/day) of A/He mice was not associated with induction of pulmonary tumors. No other organs were examined. In a 6 generation study, Kroes et al. (1977) found that the incidence of urinary bladder carcinoma was not significantly increased in Swiss SPF mice exposed to 0.2 or 0.5% saccharin diet for 21 months. It was not specified whether other tissues were examined.

A second cholesterol:saccharin (4:1) pellet implantation study in female Swiss mice significantly increased the incidence of urinary bladder carcinomas but not in the degree of malignancy in mice living more than 175 days after bladder implantation versus controls (cholesterol pellet implants only) (Bryan et al., 1970). Since all of the saccharin was removed from the implanted pellets within 1.5 days and the cholesterol plus saccharin pellet was porous, having lost 20% of its weight, it has been argued that the cholesterol:saccharin pellet was different and perhaps more irritating than the pellet comprised of only cholesterol and, furthermore, there is some concern regarding how closely pellet implantation resembles chronic oral exposure to saccharin (Cranmer, 1980).

The incidence of transitional-cell bladder cancers, lung tumors, hepatomas, or lymphomas was not significantly increased in Charles River CD mice exposed to a 1 or 5% sodium saccharin diet for up to 2 years (Homburger, 1978). Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically.

Prasad and Rai (1986) orally administered albino mice 0.5, 1.0, or 1.5 g/kg saccharin (purity not specified) dissolved in 1 mL of distilled water for 1 yr, beginning at 6 weeks of age. Papillary adenocarcinoma of the thyroid was found in male (5/10) and female (3/10) mice exposed to the highest dose. The tumors were detected during months 9-12 of the experiment and were malignant in nature; metastases were found in the lungs. No information was provided on gross or microscopic examinations of the bladder. Although a control group was used (10 males, 10 females), the tumor incidence in these mice was not reported. The saccharin used in this study was purchased from Boots Co., Bombay, India.

In female weanling BALB/c mice administered a 0, 0.1, 0.5, 1.0, or 5.0% sodium saccharin diet for 117 weeks, there was a marginally significant dose-response ($p = 0.04$) in the incidence of Harderian neoplasms (27/163, 32/172, 29/160, 22/132, and 22/84, respectively). There was no significant increase, however, for bladder, liver, breast, adrenal, or lung tumors, or for reticulum cell sarcoma or lymphoma in any dose group (Frederick et al., 1989). Neither the authors nor the NTP staff consider the Harderian gland response to be biologically significant.

4.1.3 Rats

Seven of 18, 21-day-old Osborne-Mendel rats exposed to a 5% saccharin diet for up to 2 years developed abdominal lymphosarcomas (Fitzhugh et al., 1951). The authors stated that this was not "out of line with the incidence (of abdominal lymphosarcomas) in a comparable group of rats", but noted an uncommon co-occurrence of thoracic lymphosarcomas with abdominal lymphosarcomas in 4 of the 7 rats treated for 102 or more weeks. Urinary bladders were not evaluated. Although controls were used in this study, the control tumor incidence was not provided. IARC (1980) reviewed Fitzhugh et al. (1951) and noted the small number of animals exposed.

Saccharin was negative for tumorigenesis in male and female Boots-Wistar rats exposed to a 0.005, 0.05, 0.5, or 5% saccharin diet for 2 years. Of 4 rats exposed to the highest dose and examined histologically, 1 female had a bladder papilloma (Lessel, 1971). IARC (1980) noted the small number of bladders examined histologically. It was not specified whether other tissues were examined.

There was no increase in the incidence of benign and malignant mesenchymal tumors or of bladder tumors in 70- to 90-day-old BD rats exposed to 0.2 or 0.5% sodium saccharin in the diet for up to 30 months (Schmähl, 1973; cited by IARC, 1980). It was not specified whether other tissues were examined.

In a two-generation study, the incidence of bladder cancer was not increased in F_1 male or female Charles River CD rats exposed to 0.01, 0.1, 1.0, or 5% sodium saccharin for up to 28 months. However, the incidence of urinary bladder transitional-cell neoplasms in F_1 male rats exposed to a 7.5% sodium saccharin diet for up to 28 months was significantly increased when compared to controls (7/23 vs. 1/29 in controls). In addition, there were 2/31 urinary bladder neoplasms in F_1 females exposed to 7.5% saccharin versus 0/24 in controls. The F_0 parents were fed test diets from weaning, through mating, and through gestation to the weaning of their litters. The occurrence of the bladder neoplasms was not correlated with the presence of bladder stones, and bladders were free of parasites (Taylor et al., 1980).

In a 2-generation study, there was an increased incidence of transitional-cell carcinoma of the bladder in F_1 male Sprague-Dawley rats fed 5% sodium saccharin in the diet for 100 weeks (7 tumors in 20 exposed rats vs. 0 tumors in 20 controls). Carcinomas were not observed in the bladder of female rats exposed similarly. Male and female rats fed a 0.05 or 0.5% sodium saccharin diet for 100 weeks did not show an increased incidence of neoplasms at any site (Tisdel et al., 1974).

Urinary bladder tumors were not observed in Wistar rats exposed to 2.5 g sodium saccharin/kg/day for up to 28 months (Furuya et al., 1975 abstr.). IARC (1980) noted the incomplete reporting of this study.

Sodium saccharin was negative for urinary bladder tumorigenesis in male and female weanling Charles River CD rats exposed in the diet to 90, 270, 810, or 2430 mg sodium saccharin/kg/day for 26 months. Non-invasive bladder tumors were detected in 1/60 males and 1/60 females exposed to 90 mg/kg and in 2/60 males exposed to 810 mg/kg, but none were detected in the rats exposed to 2430 mg/kg. The authors found that the presence of bladder calculi was not associated with exposure or the presence of bladder tumors. The combined incidence of lymphomas and leukemias in males given the highest dose was 7/54 (vs. 2/57 in controls), but the statistical significance of this was not specified. All major tissues were examined (Munro et al., 1975).

The incidences of tumors of the urinary bladder, pituitary, breast, and subcutaneous tissue were not increased in Charles River CD-1 rats exposed to 1 or 5% sodium saccharin for up to 2 years. The authors noted that in 33% of all examined urines, *Trichosomoides crassicauda* ova were found (Homburger, 1978). Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically.

Sodium saccharin had no significant effect on tumor incidence in Wistar SPF rats exposed to 4 g saccharin/kg body weight in the diet for 2 years. Although there was an increase in the total number of exposed males with tumors at any site (10/70 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. Sodium saccharin also had no significant effect on tumor incidence in Wistar SPF rats exposed to 2 g saccharin/kg in drinking water for 2 years. There was an increase in the total number of exposed males with tumors at any site (11/71 males vs. 1/52 male controls) in rats exposed to saccharin in drinking water, but site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically (Chowaniec and Hicks, 1979).

In a two-generation study, the incidence of benign plus malignant bladder tumors was significantly increased ($p < 0.03$) in male Sprague-Dawley rats from both the F_0 and F_1 generations (F_0: 7/38 vs. 1/36 in controls; F_1: 12/45 vs. 0/42 in controls). The F_0 generation was exposed to a 5% sodium saccharin diet for 90 days prior to mating with continued lifetime exposure (up to 142 weeks), while the F_1 pups were exposed for up to 127 weeks. The incidence of benign plus malignant bladder tumors was not statistically increased in F_0 and F_1 females and there was no increase in the incidence of tumors of other tissues in males or females. Two F_1 saccharin-dosed females, however, did have malignant urinary bladder tumors. All organs

and all grossly abnormal areas of dermal, supportive, or skeletal tissues were examined histologically (Arnold et al., 1980).

As part of an initiation/promotion study (Hoosan et al., 1980), female Wistar rats were exposed to 2 g/kg/day of sodium saccharin in drinking water or in diet. There was no increase in urinary bladder neoplasms or other tumors in rats exposed to saccharin for two years.

There was no statistically significant increase in tumor incidence in offspring of pregnant Sprague-Dawley rats administered 0.2, 1, or 5 g saccharin/kg in aqueous solution by gavage on gestation days 14, 17, and 20. Offspring were fed normal diet and observed for life (approximately 2 years) or were killed when moribund. Complete necropsies were performed. All urinary bladders and any organs with macroscopically visible abnormalities were examined histologically (Schmähl and Habs, 1980).

The incidence of urinary bladder transitional cell papilloma was significantly increased in male ACI rats administered 5% sodium saccharin in the diet for 52 weeks beginning at 6 weeks of age (9/32 vs. 0/28 in controls, $p < 0.01$). Calculi were observed in 1 rat with bladder cancer and there was a higher level of urinary $MgNH_4PO_4$ crystals in treated rats than in controls. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*, which could have enhanced cell proliferation in the bladder. The bladder, liver, and kidneys were the only tissues examined histologically. Females were not included in the study (Fukushima et al., 1983).

No tumors were detected in the bladder, liver, or kidneys of male F344, Sprague-Dawley, or Wistar rats administered 5% sodium saccharin in the diet for 52 weeks beginning at 6 weeks of age. Females were not evaluated (Fukushima et al., 1983).

In a two-generation study, administration of a mixture of 2 or 5% sodium saccharin and sodium cyclamate (1:10 ratio) in the diet of Sprague-Dawley rats was not carcinogenic. Full necropsies were performed, including evaluation of the urinary tract (Schmähl and Habs, 1984).

In a large 2-generation study, F_0 rats were started on a test diet at 6 weeks of age; F_1 rats were started on the same test diet between 28 and 38 days of age. There was a clear dose response for urinary bladder tumors in F_1 male Charles River CD rats exposed to 1.0 to 7.5% sodium saccharin in the diet for up to 30 months (1.0%, 5/658; 3.0%, 8/472; 4.0%, 12/189; 5.0%, 15/120; 6.25%, 20/120; 7.5%, 37/118; controls, 0/324) (Schoenig et al., 1985). Females were not evaluated in this study. The authors concluded a no-effect level for bladder tumors at the 1% dietary level based upon lack of statistical significance and historic control incidences at their laboratory. Following independent review of the urinary bladder lesions, Squire also concluded a no-effect level for bladder tumors at 1% (Squire, 1985). The bladder tumor incidence in rats exposed to 5% sodium saccharin only during gestation was 0/122, while that in rats exposed to 5% sodium saccharin from birth for a single generation was 12/120 (Schoenig et al., 1985). The urinary bladder, urethra, ureter, kidneys, and all gross lesions and tissue masses were examined histologically (Schoenig et al., 1985).

Bladder carcinomas and precancerous lesions were not observed in 6-week-old male analbuminemic (low level of albumin in the serum) Sprague-Dawley rats exposed to 5% sodium saccharin in the diet for 80 weeks (Homma et al., 1991). Only the bladder was examined.

4.1.4 Nonhuman Primates

Histopathological examination of urinary bladders, kidneys, and testes of surviving and deceased male and female rhesus monkeys (exposed to 20, 100, or 500 mg saccharin/kg/day in the diet for 79 months) showed no abnormal pathology (McChesney et al., 1977 abstr.; cited by IARC, 1980).

Sieber and Adamson (1978; cited by IARC, 1980) found that sodium saccharin was negative for gross neoplasia in monkeys (4 strains, not specified by IARC) exposed to 25 mg/kg/day in the diet for 9 yr. This study was ongoing in 1980.

Twenty 0 to 1-yr-old monkeys (Cynomolgus, Rhesus, and African Green were used but additional details were not provided) were exposed to 25 mg sodium saccharin/kg/day by mouth in water for at least 20 yr. Five monkeys died from either varicella, pneumonia, or unknown reasons. No tumors were found in the dead monkeys nor were there any indications of tumors in the 15 surviving monkeys. Complete necropsies were performed on all animals that died. Various unspecified hematological and biochemical tests were routinely performed on survivors (Thorgeirsson et al., 1994).

Results from the surviving monkeys from the Thorgeirsson et al. (1994) study were subsequently reported (Cohen et al., 1996 abstr.). There were no calculi, unusual crystals, increased crystalluria, or calcium phosphate precipitate in urine of cynomolgus and rhesus monkeys administered 25 mg sodium saccharin/kg/day for 17 to 23 years. Urine was analyzed during the last year of life. There was no association between ingestion of sodium saccharin and urinary protein content. Urinary bladders were free of hyperplasia and tumors and scanning electron microscopy revealed no difference in the appearance of the urothelium in exposed and age-matched control monkeys (Cohen et al., 1996 abstr.). It was not specified in the abstract whether other tissues were examined.

Table 4-1. Mammalian Carcinogenicity

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.1 Hamsters							
8-wk-old Syrian golden hamsters	30M, 30F	none	sodium saccharin made by Maumee process, purity not specified	0.156-1.25% in drinking water	50-60 wk	Negative No urinary tract neoplasms were observed. The incidence of other neoplasms was within the range of spontaneously occurring tumors.	Althoff et al. (1975)
4.1.2 Mice							
'S' strain mice (age not specified)	20 (sex not specified)	19 (sex not specified)	saccharin[a] made by Remsen-Fahlberg method, purity not specified	8% solution in acetone, applied to skin	22 wk	Negative Twenty-five days after application of saccharin, animals were given 18 weekly applications of 0.17% croton oil in acetone. Following treatment with croton oil, 14 skin tumors were observed in 7/20 animals exposed to saccharin, while 4 skin tumors were observed in 4/19 control animals treated with croton oil only. This difference was not significant (p value not given).	Salaman and Roe (1956; cited by IARC, 1980)
"stock' mice (age not specified)	20 (sex not specified)	28 (sex not specified)	saccharin[a], method of production and purity not specified	2 mg saccharin/8 mg cholesterol pellets	40 or 52 wk	Positive Saccharin/cholesterol pellets were implanted in urinary bladder lumina. Controls received cholesterol pellets. Of mice which survived for at least 30 weeks, 4/13 saccharin-treated mice and 1/24 control mice had bladder cancer ($p=0.01$; χ^2 test). The authors noted that the presence of the cholesterol pellet in the bladder may have had a promoting action and that the method of bladder implantation detects incomplete carcinogens. It was not specified whether other tissues were examined.	Allen et al. (1957)
60- to 90-day-oldSwiss mice	100F	100F	sodium saccharin; method of production and purity not specified	20-24 mg pellets with 20% sodium saccharin suspended in cholesterol	13 mo	Positive Saccharin/cholesterol pellets were implanted into the urinary bladder lumina. Controls received cholesterol pellets. Incidences of mouse bladder carcinomas in exposed animals were 47 and 52% as compared with incidences of 13 and 12% in controls. The time required for 50% of the compound to be eluted was about 5.5 hours, so the exposure of the mouse bladder to saccharin was very brief.	Bryan et al. (1970)
Swiss mice (age not specified)	50F	50F	saccharin[a], method of production and purity not specified	5% in diet	18 mo	Negative Saccharin did not alter incidence of tumors (type not specified) and did not affect urinary bladder pathology when bladder was observed macroscopically. It was not specified which other tissues were examined.	Roe et al. (1970)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
A/He mice (age not specified)	20F per dose	30F	saccharin[a] method of production and purity not specified	0.6 or 3.3 g/kg/day i.p.	8 wk	Negative Exposed animals were killed after 21 weeks, controls were killed after 24 weeks. Exposure to saccharin was not associated with induction of pulmonary tumors. The lungs were the only tissue examined.	Stoner et al. (1973; cited by IARC, 1980)
Swiss SPF mice (age not specified)	50M, 50F per dose	50M, 50F	saccharin[a], made by Remsen-Fahlberg method, 0.5% *o*-toluenesulfonamide, impurity	0.2 or 0.5% in diet (6-generation study)	21 mo	Negative Exposure to saccharin did not significantly alter the incidence of urinary bladder carcinoma. It was not specified whether other tissues were examined.	Kroes et al. (1977)
Charles River CD mice (age not specified)	25M, 25F per dose	25M, 25F	sodium saccharin, method of production not specified, 345 mg/kg *o*-toluenesulfonamide	1 or 5%	≤ 2 yr	Negative Animals were sacrificed when obvious tumors were seen or when they were moribund. Survivors were killed at 2 years. Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically. The incidence of transitional-cell bladder cancers in treated animals was not significantly different from that in controls. Lung tumors, hepatomas, and lymphomas occurred with similar frequency in exposed and control animals. This study was complicated by the presence of the worm *Trichosomoides crassicauda* in treated and control animals. The author stated that this parasite is known to cause extensive papillomatosis of the bladder.	Homburger (1978)
6-wk-old albino mice	10M, 10F per dose	10M, 10F	saccharin[a], method of production and purity not specified (purchased from Boots Co., Bombay, India)	0.5, 1.0, or 1.5 g/kg/day in 1 mL distilled water, by gavage (times/wk not specified)	1 yr	Positive Papillary adenocarcinoma of the thyroid was found in male (5/10) and female (3/10) mice exposed to the highest dose. The tumors were detected during months 9-12 of the experiment and were malignant in nature; metastatic deposits were found in the lungs. No information was provided on gross or microscopic examinations of the bladder. The tumor incidence in controls was not reported.	Prasad and Rai (1986)
18- to 19-wk-old BALB/c mice	192F (0.1%) 192F (0.5%) 144F (1.0%) 96F (5.0%)	192F (basal diet alone)	sodium saccharin, >98% pure, method of production not specified	0, 0.1, 0.5, 1.0, or 5.0% diet	117 wk	Negative There was a marginally significant trend (p=0.04) in the incidence of Harderian neoplasms (27/163, 32/172, 29/160, 22/132, 22/84). There was no significant dose-response for bladder, liver, breast, adrenal, or lung tumors, or for reticulum cell sarcoma or lymphoma in any dose group. The Harderian gland response was not considered to be biologically significant.	Frederick et al. (1989)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.3 Rats							
21-day-old Osborne-Mendel rats	10M, 10F per dose	10M, 10F	saccharin[b], method of production and purity not specified	0.01, 0.1, 0.5, 1, or 5% in diet	≤ 2 yr	Negative Seven of 18 animals (sex not specified) receiving 5% dose developed abdominal lymphosarcomas. The authors stated that this was not "out of line with the incidence (of abdominal lymphosarcomas) in a comparable group of rats", but noted the uncommon co-occurrence of thoracic lymphosarcomas with abdominal lymphosarcomas in 4 of the 7 rats treated for 102 or more weeks. Tumor incidence in controls was not provided. Urinary bladders were not evaluated. IARC (1980) noted the small number of animals used in this study.	Fitzhugh et al. (1951)
Boots-Wistar rats	20M, 20F per dose	20M, 20F	saccharin[a], made by Remsen-Fahlberg method, purity not specified	0.005, 0.05, or 5% in diet	2 yr	Negative Tumor incidence was similar in control and exposed animals. Of 5 bladders from animals exposed to the highest dose, 1 female had a bladder papilloma. IARC (1980) noted the small number of bladders examined histologically. It was not specified whether other tissues were examined.	Lessel (1971)
70- to 90-day-old BD rats	52M, 52F per dose	52M, 52F	sodium saccharin, made by Remsen-Fahlberg method, purity not specified	0.2 or 0.5% in diet	≤ 30 mo	Negative The incidence of benign and malignant tumors was similar in control and exposed animals. No bladder tumors were observed. *Strongyloides capillaria* was found in the urinary tract of 16% of all animals. [original paper in German]	Schmähl (1973)
weanling SD rats	20M, 20F per dose	20M, 20F	sodium saccharin, made by Remsen-Fahlberg method, purity not specified	0.05, 0.5, or 5% in diet	100 wk	Positive (males only, at highest dose) F_0 generation was fed same dose as offspring. There were seven transitional-cell carcinomas of the urinary bladder, but only in males fed the highest dose. A review by IARC (1980) noted that this incidence was significant (p=0.001).	Tisdel et al. (1974)
Wistar rats (age not specified)	54-56M	54-56M	sodium saccharin, method of production and purity not specified	2.5 g/kg body weight/day	≤ 28 mo	Negative No urinary bladder tumors were observed. It was not specified whether other tissues were examined. IARC noted the incomplete reporting of this study.	Furuya et al. (1975 abstr.)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Weanling Charles River CD rats	240M, 240F	60M, 60F	sodium saccharin, method of production and purity not specified	90, 270, 810 or 2430 mg/kg/day in diet	26 mo	Negative Non-invasive bladder tumors were detected in 1/60 males and 1/60 females exposed to 90 mg/kg and in 2/60 males exposed to 810 mg/kg, but not in any rats exposed to 2430 mg/kg. The presence of bladder calculus was not associated with exposure or with the presence of bladder tumors. Saccharin administration was not accompanied by an increase in tumor incidence. The combined incidence of lymphomas and leukemias in males given the highest dose was 7/54 (vs. 2/57 controls), but the statistical significance of this was not specified. All major tissues were examined.	Munro et al. (1975)
Charles River CD-1 rats (age not specified)	25M, 25F per dose	25M, 25F	sodium saccharin, method of production not specified, 345 mg/kg *o*-toluenesulfonamide	1 or 5% in diet	≤ 2 yr	Negative Animals were sacrificed when obvious tumors were seen or when they were moribund. Survivors were killed at 2 yr. Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically. The incidences of tumors of the urinary bladder, pituitary, breast, and subcutaneous tissue were similar in control and exposed animals. The author stated that one third of all examined urines were thought to contain *Trichosomoides crassicauda* ova.	Homburger (1978)
Wistar SPF rats (age not specified)	75M, 75F	55M, 50F (these controls also used for drinking water study; see below)	sodium saccharin, made by Remsen-Fahlberg method, 698 mg/kg *o*-toluenesulfonamide	4 g/kg body weight; in diet	2 yr	Negative Although there was an increase in the total number of exposed males with tumors at any site (10/70 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically.	Chowaniec and Hicks (1979)
Wistar SPF rats (age not specified)	75M, 50F	55M, 50F	sodium saccharin, made by Remsen-Fahlberg method, 698 mg/kg *o*-toluenesulfonamide	2 g/kg body weight; in drinking water	2 yr	Negative Although there was an increase in the total number of exposed males with tumors at any site (11/71 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically.	Chowaniec and Hicks (1979)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
32-day-old SD rats	50M, 50F	50M, 50F	sodium saccharin, made by Maumee process, < 0.05 ppm *o*-toluenesulfonamide	5% in diet	90 days (adults), ~700 days (pups)	Positive (only males) Incidence of benign plus malignant bladder tumors was significantly increased ($p < 0.03$) in exposed male rats from both the F_0 and F_1 generations (F_0: 7/38 vs. 1/36 controls; F_1: 12/45 vs. 0/42 controls). The incidence of benign plus malignant bladder tumors was not statistically increased in F_0 or F_1 females and there was no increase in the incidence of tumors of other tissues in males or females. All organs and all grossly abnormal areas of dermal, supportive, or skeletal tissues were examined histologically. There were no effects on reproduction, longevity, or hematological parameters.	Arnold et al. (1980)
Wistar rats (age not specified)	50F	63F	sodium saccharin, made by Maumee process, purity not specified	2 g/kg body weight/day	2 yr	Negative No bladder neoplasms occurred in control or exposed rats. Overall tumor incidence did not differ between control and exposed rats. It was not specified in the review which tissues besides bladders were examined. IARC noted that animals were started on the test diet not at weaning, but after several weeks on a normal diet.	Hooson et al. (1980; cited by IARC, 1980)
pregnant SD rat	5F (low dose) 6F (mid dose) 7F (high dose)	5F	saccharin[b] [< 10 ppm *o*-toluene sulfonamide], method of production not specified	0.2, 1, or 5 g/kg by gavage in aqueous solution, administered on gestation days 14, 17, and 20	3 days	Negative There was no statistically significant increase in tumor incidence in offspring that were fed normal diet and observed for life (~ 2 yr) or were killed when moribund, as compared to offspring of controls. Complete necropsies were performed. All urinary bladders and any organs with macroscopically visible abnormalities were examined histologically.	Schmähl and Habs (1980)
in utero Charles River CD rats	240M, 240F	48M, 48F	sodium saccharin, made by Remsen-Fahlberg method, 350 ppm *o*-toluenesulfonamide	0.01, 0.1, 1, 5, or 7.5% in diet	≤ 2 yr	Positive in males at highest dose. There was an increased incidence of urinary bladder tumors in F_1 males fed 7.5% sodium saccharin (7/23 vs. 1/29 controls). F_0 rats were fed test diet continuously from weaning through mating, and through gestation to weaning of their litters. Complete necropsies were performed. The urinary bladder, all tumors, and any grossly abnormal tissues were examined histologically.	Taylor et al. (1980)
6-wk-old ACI rats	48M	45M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Positive The incidence of urinary bladder transitional cell papilloma was significantly increased at 52 wk (9/32 vs. 0/28 controls, $p< 0.01$). Calculi were observed in 1 rat with bladder cancer and there was a higher level of urinary "crystals" in treated rats than in controls. The bladder, liver, and kidneys were the only tissues examined histologically. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*.	Fukushima et al. (1983)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old F344 rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
6-wk-old SD rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
6-wk-old Wistar rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
newborn SD rats	33M, 39F (low dose) 34M, 37F (high dose)	36M, 34F	sodium saccharin [0.0005% *o*-toluene sulfonimide] and sodium cyclamate, method of production not specified	2 or 5% sodium saccharin and sodium cyclamate in the diet (1:10 ratio)	lifetime (parents were also fed same dose)	Negative The mixture of sodium saccharin and sodium cyclamate was not carcinogenic at either dose. Detailed necropsies were performed, including evaluation of the urinary tract.	Schmähl and Habs (1984)
28- to 38-day-old Charles River CD rats	1%, 700M; 3%, 500M; 4%, 200M; 5%, 125M; 6.25%, 125M; 7.5%, 125M; 5% (through gestation), 125M; 5% (following gestation), 125M	350M	sodium saccharin, made by Maumee process, >99% pure	1.0, 3.0, 4.0, 5.0, 6.25, or 7.5% in diet (same dose used for parent and offspring)	30 mo	Positive Parents were exposed to same dose from 6 weeks of age. A clear dose response for urinary bladder tumors was observed in F_1 male rats (1.0%, 5/658; 3.0%, 8/472; 4.0%, 12/189; 5.0%, 15/120; 6.25%, 20/120; 7.5%, 37/118; all vs. 0/324 in controls). Female F_1 rats were not evaluated. Tumor incidence in rats exposed only to 5% sodium saccharin during gestation was similar to controls. 12/120 rats exposed to 5% sodium saccharin from birth for a single generation had bladder tumors. The urinary bladder, urethra, ureter, kidneys, and all gross lesions and tissue masses were examined histologically.	Schoenig et al. (1985)
6-wk-old analbuminemic and SD rats	35M analbuminemi 36M SD	12M analbuminemic, 14M SD	sodium saccharin, method of production and purity not specified	5% in diet	80 wk	Negative No bladder carcinomas or precancerous lesions were observed in any of the rats. Only the bladder was examined.	Homma et al. (1991)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.4 Nonhuman Primates							
rhesus monkeys (age not specified)	7M, 7F	3M, 3F	sodium saccharin, method of production not specified, 'purified'	20, 100, or 500 mg/kg/day in diet	79 mo	Negative Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology.	McChesney et al. (1977 abstr.; cited by IARC, 1980)
monkeys (4 unspecified strains)	10 total	0	sodium saccharin, method of production not specified, 'purified'	25 mg/kg/day in diet	9 yr	Negative Clinical observation revealed no gross neoplasia. This study was ongoing in 1980.	Sieber and Adamson (1978; cited by IARC, 1980)
0 to 1-yr-old monkeys (Cynomolgus Rhesus, and African Green)	20 total	0	sodium saccharin, method of production and purity not specified	25 mg/kg/day in diet	>20 yr	Negative Dose corresponds to 5 cans diet soda per day by 70 kg human. Five monkeys died from either varicella, pneumonia, or unknown reasons. No tumors were found in the dead or in any of the 15 surviving monkeys. Complete necropsies were performed on all animals that died. Various unspecified hematological and biochemical tests were routinely performed on survivors.	Thorgeirsson et al. (1994)
Cynomolgus and Rhesus monkeys (age not specified)	not specified	not specified	sodium saccharin, method of production and purity not specified	25 mg/kg/day in diet	17-23 yr	Negative Urine was analyzed during last year of life. There were no calculi, unusual crystals, increased crystalluria, or calcium phosphate precipitate in urine. There was no association between ingestion of sodium saccharin and urinary protein content. Urinary bladders were free of hyperplasia and tumors. There was no difference in appearance of urothelium in exposed and age-matched control monkeys. It was not specified whether other tissues were examined.	Cohen et al. (1996 abstr.)

Abbreviations: F = females; i.p. = intraperitoneally; M = males;

[a] No distinction was made between saccharin and its sodium salt in the IARC discussion

[b] No distinction was made between saccharin and its sodium salt

4.2 Initiation/Promotion and Co-Carcinogenicity Studies

Experimental details for the studies described in this section are presented in **Table 4-2**.

4.2.1 Benzo[*a*]pyrene (BP)

Saccharin did not enhance the incidence of tumors in the forestomach of mice exposed to a test diet containing 5% saccharin (for 72 wk) starting 7 days after an initial single gastric instillation of 0.2 mL polyethylene glycol containing 50 µg BP. No pathological changes were observed macroscopically in urinary bladders of saccharin-exposed mice (Roe et al., 1970). It was noted that BP is not organotropic for the bladder and that histological examination of the urinary bladders was not conducted (IARC, 1980).

4.2.2 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)

Sodium saccharin, administered in the diet at 0.04, 0.2, 1, or 5% for 32 wk, did not produce any effects in 6-wk-old Charles River F344 rats that were not pretreated with BBN (0.01% in water for 4 wk). A sodium saccharin dose-dependent increase in papillary or nodular hyperplasia of the urinary bladder was statistically significant in females (1% and 5% sodium saccharin groups) and males (5% sodium saccharin group) in the BBN-exposed groups (Nakanishi et al., 1980a).

The effects of sequential administration (initiation/promotion protocol) of 0.01% BBN in drinking water and 5.0% sodium saccharin in feed and co-administration of 0.001% BBN in drinking water and 5.0% sodium saccharin in feed, were studied in 8-wk-old male Wistar rats by Nakanishi et al. (1980b). In the first experiment (sequential administration), rats received BBN for 4 wk and then sodium saccharin for an additional 32 wk. In the second experiment (co-administration), rats were fed BBN and sodium saccharin for 40 wk. When rats were administered BBN and sodium saccharin concurrently, there was an increased incidence of urinary bladder papilloma (10/24 vs. 0/12 in controls). Sequential administration produced a non-statistically significant increase (9/31 vs. 0/12 in controls) in the incidence of bladder papilloma. In addition, there was one transitional cell carcinoma among the 31 rats that received saccharin sequentially and two transitional cell carcinomas among the 24 rats receiving saccharin concurrently with BBN. Transitional cell hyperplasia was noted in rats receiving sodium saccharin alone as well as in groups receiving BBN before and during saccharin administration.

Nakanishi et al. (1982) reported that there was no statistically significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma in male F344 rats (age not specified) initiated with 0.01% BBN in drinking water for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone. Sodium saccharin significantly enhanced urothelial hyperplasia after BBN pretreatment and produced a non-statistically significant increase in urinary bladder papillomas (6/29 vs. 0/29 in controls).

The comparative tumor-promoting effects of 5% sodium saccharin, 5% sodium L-ascorbate, 5% L-ascorbic acid, 5% sodium saccharin plus sodium L-ascorbate, or 5% sodium saccharin plus L-ascorbic acid were studied in 6-wk-old male F344 rats. Rats were initiated with 0.05% BBN in drinking water for 4 wk and were then fed the test diets for an additional 32 wk. The authors found that bladder-cancer promotion by sodium saccharin was inhibited by L-

ascorbic acid and enhanced by sodium L-ascorbate, apparently as a function of urinary pH. Sodium saccharin alone, sodium L-ascorbate alone, and these two compounds in combination caused increased incidences of urothelial hyperplasia, papilloma, and carcinoma in the urinary bladder (Fukushima et al., 1990).

Yu et al. (1992) studied the tumor-promoting effects of sodium saccharin alone and in combination with nordihydroguaiaretic acid (an antioxidant and inhibitor of arachidonic acid metabolism) in BBN-initiated male F344 rats. BBN (0.05%) was administered to 6-wk-old rats in the drinking water for 4 wk. The rats were then fed 5% sodium saccharin with or without the antioxidant for an additional 36 wk. Nordihydroguaiaretic acid was coadministered at a concentration of 0.1% in the diet. The authors found that sodium saccharin promoted BBN tumorigenicity, while nordihydroguaiaretic acid plus sodium saccharin decreased the incidences of papilloma. Both groups receiving sodium saccharin had urothelial hyperplasia.

4.2.3 2-Acetylaminofluorene (AAF)

Nakanishi et al. (1982) reported that there was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder carcinoma in male F344 rats (age not specified) initiated with 0.02% AAF in the diet for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered AAF alone. There was a statistically significant increase in urothelial hyperplasia in the sodium saccharin-promoted rats.

The effect of lifetime sodium saccharin dosing (0.1, 0.5, 1.0, or 5.0% diet for 117 wk), administered 2 wk after initiation with AAF (200 ppm diet for 13 weeks), on female weanling BALB/c mice was studied by Frederick et al. (1989). No dose-related increase in tumor incidence was found in initiated mice exposed to 0.1-5% sodium saccharin diet.

In female Horton SD rats (age not specified) co-administered 300 mg AAF/kg diet and 5% sodium saccharin in the diet for 40 wk, no animals developed malignant lesions of the urinary bladder. Eleven of the 12 AAF-treated rats (no sodium saccharin in diet) developed palpable mammary and ear-duct tumors, while 6/12 animals exposed to AAF and sodium saccharin developed these tumors. Liver tumors occurred in control and exposed animals (Ershoff and Bajwa, 1974; cited by IARC, 1980). IARC (1980) noted that the small number of animals used and the fact that food consumption was not measured prevented the evaluation of AAF and sodium saccharin exposure.

4.2.4 *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT)

The effects of sodium saccharin in FANFT-initiated (0.2% diet for 6 wk) 4-wk-old male F344 rats were studied by Cohen et al. (1979). Subsequent to initiation with FANFT, rats were exposed to a 5% sodium saccharin diet (*o*-toluenesulfonamide free) for up to 83 wk. Two other groups received *o*-toluenesulfonamide-free sodium saccharin either with or without FANFT initiation following a 6-wk no-exposure period. The incidence of bladder cancer was not increased in the sodium saccharin-only group (0/20) when compared to the no-exposure control group (0/42). In the FANFT-initiated control group, 4/20 rats developed bladder cancer and 1/20 rats developed bladder papilloma. In the FANFT plus sodium saccharin groups with or without a 6-wk no-exposure period, the incidences of bladder cancer were 13/18 and 18/19, respectively.

Fukushima et al. (1981) fed 5-wk-old male F344 rats 0.2% FANFT diet for only a 4-wk initiation period in order to decrease the production of bladder cancer in the FANFT-only group. Rats were subsequently fed a 5% sodium saccharin or control diet for 100 wk. There was a significant increase ($p < 0.03$) in the incidence of carcinoma of the bladder as compared to FANFT-only controls (5/26 vs. 0/25).

Murasaki and Cohen (1983a) evaluated the co-carcinogenicity of FANFT (0.005% diet) and sodium saccharin (5% diet) administered to 5-wk-old male Fischer rats for 2 yr. The authors reported that the incidence of bladder lesions was marginally significant ($p < 0.06$), when compared to the incidence in FANFT-only controls (5/16 vs. 0/11). There were no statistically significant increases in tumor incidences for other tissues.

Imaida and Wang (1986) studied sodium saccharin as a promoter in a two-stage carcinogenesis model. Groups of 42 or 43 male weanling F344 rats were exposed to 5% sodium saccharin in AIN-76A diet for 100 wk subsequent to a 4-wk regimen of exposure to either 0.2% FANFT in Wayne diet or 0.005% *N,N*-dibutylnitrosamine (DBN) in drinking water and control Wayne diet. None of the control rats fed sodium saccharin alone developed bladder, liver, esophageal, or forestomach tumors. There was no statistically significant increase in the incidence of tumors of the bladder, liver, or esophagus in rats initiated with FANFT or DBN, with a subsequent dietary administration of sodium saccharin, as compared to FANFT-only and DBN-only controls, respectively. However, the group receiving FANFT initiation followed by sodium saccharin promotion did have an increased incidence of urinary bladder carcinomas ($p = 0.059$).

The comparative effects of different chemical forms of saccharin and ascorbate in conjunction with other chemicals that would affect the urinary ionic composition and pH were studied by Cohen et al. (1991b). Rats (5-wk-old male F344) were exposed to 0.02% FANFT or control diet for 6 wk. Subsequent to administration of FANFT, rats were exposed to 3 or 5% sodium saccharin, 3.12 or 5.2% calcium saccharin, 2.53 or 4.21% acid saccharin, 4.44% ascorbic acid, or 5% sodium ascorbate diet for 72 wk. Carcinomas and papillomas developed in 12/39 (31%) and 5/39 (13%) rats, respectively, in the FANFT-only group. A statistically significant increased incidence of tumorigenesis occurred in all of the other groups, with the exception of acid saccharin, ascorbic acid, and low-dose calcium saccharin. Sodium saccharin > sodium ascorbate > calcium saccharin for enhancement of bladder tumorigenesis; none of the forms of saccharin were tumorigenic without FANFT initiation. The authors found that an elevated urinary pH increased tumorigenicity. However, elevated urinary sodium concentrations are sufficient, as shown by the enhancement of bladder tumor promotion by sodium saccharin and sodium ascorbate, and by the enhanced bladder tumorigenicity of calcium saccharin when sodium chloride was added to the calcium saccharin exposure. Masui et al. (1991) analyzed the tumors in this study for H-*ras* mutations by Western blotting using a monoclonal antibody against p21. H-*ras* mutations were found in 2/3 and 3/6 bladder tumors from rats exposed to FANFT alone, and 4/20 and 1/10 H-*ras* mutations were found in tumors from rats exposed to FANFT initiation with 3 or 5% sodium saccharin promotion, respectively.

Okamura et al. (1991) compared the Prolab 3200 with the AIN-76A diet for the promoting effects of sodium saccharin and found that male F344 rats on Prolab 3200 diet exhibited sodium saccharin (5% diet for 100 wk) enhancement of bladder tumors when initiated

for 4 wk with 0.2% FANFT. This effect was not found in the AIN-76A-fed rats initiated with FANFT and fed 5% sodium saccharin for 100 wk.

4.2.5 *N*-Methyl-*N*-nitrosourea (MNU)

A series of reports on studies conducted by Hicks et al. (1973, 1975) and Hicks and Chowaniec (1977) evaluated sodium saccharin (2-yr exposure) following intravesicular instillation of MNU (single dose of 1.5 or 2 mg) in 6- to 8-wk-old Wistar rats. In 138 rats in the male and female 4 g/kg/day dietary sodium saccharin-only group, 3 bladder tumors were found. Administration of 2 g/kg/day of sodium saccharin in drinking water for two yr did not produce any bladder tumors in male and female Wistar rats. Bladder tumors were found in 23/49 (47%) female rats in an MNU plus 2 g/kg/day dietary sodium saccharin group. Bladder tumor incidence was increased in the MNU plus 4 g/kg/day sodium saccharin female group (27/47; 57%).

In an effort to reproduce the experiments of Hicks et al. (1973, 1975) and Hicks and Chowaniec (1977), Mohr et al. (1978) instilled 2 mg MNU in the bladders of female Wistar/AF-Han rats which were subsequently fed 2% sodium saccharin for the first 10 wk and 4% afterwards [up to 2 yr] (specific dosing regimen not specified). In the MNU-only group, bladder tumors were found in 19/49 (39%) rats; and ureter tumors were found in 8/49 (17%) rats, while 14/49 (28%) rats developed renal pelvis tumors. In the MNU plus sodium saccharin group, incidences of renal pelvis, ureter, and bladder tumors were 43, 11, and 39%, respectively. The high incidence of tumors in the MNU-only group was explained by the original authors as a result of the use of MNU within 15 min of dissolution and the assumption that in their experiment the dose of MNU was not subcarcinogenic.

Hooson et al. (1980) studied the contribution of the sodium saccharin contaminant *o*-toluenesulfonamide in the promotion of MNU-initiated bladder carcinogenesis in female Wistar rats (age not specified). No statistically significant differences were found in bladder tumor incidence with administration of a single 0.15 mL-dose of MNU, followed 2 wk later by daily administration in drinking water or diet of either 2 g/kg *o*-toluenesulfonamide-free sodium saccharin or 2 g/kg sodium saccharin containing 40 mg/kg *o*-toluenesulfonamide for 2 yr, as compared to a control group given MNU alone. There was, however, a decrease in the latency period in the MNU+sodium saccharin treated groups (55 and 52 wk vs. 87 wk for MNU-only controls).

West et al. (1986) exposed 8-wk-old female Sprague-Dawley rats, which had previously been dosed with a single dose of MNU or by saline transurethral instillation into the bladder, to 0.1, 0.5, 1.0, 2.5, or 5% sodium saccharin in the diet. Other groups received MNU followed by 2% sodium saccharin in water or 5% acid saccharin diet. Sodium saccharin dosing was initiated 2 days after rats were dosed with MNU and continued until the termination at 102 wk. In MNU-exposed rats, histopathological examination revealed papillomas and carcinomas of the urinary bladder. A mortality-adjusted increase in tumor incidence and a decrease in time-to-tumor with increasing sodium saccharin dose for the 0-2.5% doses in dead and moribund rats was reported. A statistically significant increase in bladder tumor prevalence ($p < 0.0012$) was found for the group of rats exposed to 2.5% sodium saccharin plus MNU vs. the MNU-only control group. The greatest number of tumors developed in rats that received four doses of MNU alone throughout the experiment. Rats not exposed to MNU that were dosed with a 0.1-5% sodium

saccharin or 5% acid saccharin diet or 2% sodium saccharin in the drinking water developed a small number of tumors that were not significantly different from controls.

In the bladders of female Sprague-Dawley rats exposed to 1.0, 2.5, or 5.0% dietary sodium saccharin (given 4 wk immediately preceding, following, or centered on the day of bladder instillation of 0.5mg MNU), MNU-induced tumorigenesis was not enhanced (West et al., 1994). After the 4-wk administration of dietary sodium saccharin, rats were maintained on control diet. Additional groups of rats were dosed neonatally in the milk by administration of the same three levels of dietary saccharin to the dams during three wk of lactation. These latter groups then received MNU by bladder instillation at 8 wk of age and remained on the control diet for up to 106 wk of age. This neonatal exposure to saccharin did not enhance MNU-induced bladder tumors.

4.2.6 Freeze Ulceration

Five-wk-old male F344 rats had their bladder cells initiated with application of a steel rod frozen in dry ice and acetone (freeze ulceration). Rats were subsequently fed a control diet for two wk and then a sodium saccharin diet for 102 wk. This treatment resulted in 5/20 (25%) carcinomas and 1/20 (5%) papillomas compared with none when rats were exposed to either dosage regimen (freeze ulceration or sodium saccharin diet) alone. When 0.2% FANFT was administered in the diet for 2 wk after freeze ulceration followed by 5% sodium saccharin diet for 102 wk, 4/23 (17%) of the bladders had tumors. Reversing the order of FANFT and freeze ulceration exposure resulted in an 8/22 (36%) incidence of bladder tumors. Tumors were not found in rats that had received FANFT or sodium saccharin alone (Cohen et al., 1982).

Hasegawa et al. (1985) fed 5-wk-old male F344 rats 5% sodium saccharin diet either immediately or 2, 4, 6, or 18 wk following freeze ulceration of the bladder. There was a significant increase in the incidence of transitional cell carcinoma of the bladder in all of these groups as compared to a freeze ulceration-only control group, except in the group fed sodium saccharin 2 wk after freeze ulceration (11/36, 6/36, 12/40, 7/36, 9/39 vs. 1/39 in controls). No bladder carcinoma was detected in control rats administered saccharin alone.

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.1 Benzo[*a*]pyrene (BP)							
Swiss mice (age not specified)	50F	50F	saccharin[a], method of production and purity not specified	5% diet	18 mo	Negative Animals were gavaged with a single 0.2 mL dose of polyethylene glycol, either alone or containing 50 µg BP. Seven days after BP treatment, exposure to saccharin was begun. BP induced an increased incidence of tumors of the forestomach but saccharin did not enhance this increase. No pathological changes were observed macroscopically in urinary bladders of saccharin-exposed mice. IARC (1980) noted that BP is not organotropic for the bladder and that a histological examination of the urinary bladders was not done.	Roe et al. (1970)
4.2.2 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)							
6-wk-old Charles River F344 rats	30M, 31F	30M, 31F	sodium saccharin	0.04, 0.2, 1, or 5% diet	32 wk	Positive Rats were preexposed to 0.01% BBN in water for 4 wk. Sodium saccharin did not produce any effects in rats that were not preexposed to BBN. In the BBN exposed groups, a sodium saccharin dose-dependent increase in papillary or nodular hyperplasia of the urinary bladder achieved statistical significance in females (1% and 5% sodium saccharin) and males (5% sodium saccharin).	Nakanishi et al. (1980a)
8-wk-old Wistar rats	40M (BBN/ sodium saccharin)	24M (BBN alone) 24M (sodium saccharin alone) 18M (no chemicals)	sodium saccharin, >99.5% pure, 7 ppm *o*-toluenesulfon-amide	sodium saccharin: 5% diet 0.01% in drinking water	Rats pretreated with BBN for 4 wk and then given sodium saccharin for 32 wk	Negative for urinary bladder cancer There was no statistically significant increase in the incidence of urinary bladder cancer. There was an increased incidence of urinary bladder papillomas (10/24 vs. 0/12 in controls).	Nakanishi et al. (1980b)
F344 rats (age not specified)	31M	30M (BBN alone)	sodium saccharin, 7 ppm *o*-toluenesulfonamide; method of production and purity not specified	0.01% BBN in drinking water for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Negative There was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma.	Nakanishi et al. (1982)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old F344 rat	15-16M/ dose group	15-16M (BBN alone)	sodium saccharin sodium L-ascorbate L-ascorbic acid sodium saccharin plus sodium L-ascorbate sodium saccharin plus L-ascorbic acid Methods of production and purities not specified	5% diet 5% diet 5% diet 5% diet 5% diet	32 wk (All rats were administered drinking water containing 0.05% BBN for 4 wk and were then given test diet or control diet for an additional 32 wk)	Positive with BBN pretreatment. When administered individually following BBN initiation, sodium saccharin and sodium L-ascorbate significantly increased the incidence of urinary bladder hyperplasia (14/15 and 15/16), papilloma (9/15 and 13/16), and carcinoma (5/15 and 11/16) versus BBN controls (4/15, 4/15, and 0/15). Co-administration of sodium saccharin and sodium L-ascorbate also significantly increased the incidences of bladder hyperplasia, bladder papilloma, and bladder carcinoma. These increased incidences were accompanied by increases in urinary sodium ion concentration and pH. Co-administration of sodium saccharin and L-ascorbic acid caused a decrease in urinary pH and no change in urinary sodium ion levels, and did not increase the incidence of hyperplasia, papilloma, or carcinoma.	Fukushima et al. (1990)
6-wk-old F344 rats	23M (BBN + sodium saccharin) 23M (BBN + sodium saccharin + nordihydro-guaiaretic acid	11M (sodium saccharin alone) 11M (sodium saccharin + nordihydro-guaiaretic acid) 11M (nordihydro-guaiaretic acid alone) 20M (BBN alone) 20M (BBN + nordihydro-guaiaretic acid	sodium saccharin nordihydroguaiaretic acid BBN Methods of production and purity not specified	5% diet 0.1% diet 0.05% in drinking water	36 wk	Positive for tumor promotion with 4-wk BBN pretreatment. Incidences of urinary bladder urothelial hyperplasia and papilloma were increased in sodium saccharin-treated rats versus controls. Incidences of papillary or nodular hyperplasia and papilloma were decreased by nordihydroguaiaretic acid (antioxidant; inhibitor or arachidonic acid metabolism) alone or in combination with sodium saccharin compared with sodium saccharin alone.	Yu et al. (1992)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.3 2-Acetylaminofluorene (AAF)							
21- to 26-day-old BALB/c mice	saccharin dose in parentheses 192F (0.1%) 192F (0.5%) 144F (1.0%) 96F (5.0%)	192F (AAF alone)	sodium saccharin, >98% pure, method of production not specified.	sodium saccharin: 0, 0.1, 0.5, 1.0 or 5.0% diet AAF: 200 ppm diet	13 wk initiation with AAF 2 wk control diet; 117 wk sodium saccharin diet (132 wk total)	Negative Sodium saccharin had no effect on the urinary bladder tumorigenic response of initiated mice. An increased trend (p=0.04) of Harderian gland neoplasms was not considered to represent a positive tumorigenic response.	Frederick et al. (1989)
Horton SD rats (age not specified)	62F	62F	sodium saccharin, method of production and purity not specified	5% diet	40 wk	Negative for tumorigenesis with co-administration of AAF All animals were fed 300 mg AAF/kg diet for the duration of the study. Eleven of the 12 controls (no sodium saccharin in diet) developed palpable mammary and ear-duct tumors, while 6/12 animals exposed to AAF and sodium saccharin developed these tumors. Liver tumors occurred in control and exposed animals. No animals had malignant lesions of the urinary bladder. IARC noted the small number of animals used and the fact that food consumption was not measured, preventing the evaluation of AAF and sodium saccharin exposure.	Ershoff and Bajwa (1974; cited by IARC, 1980)
F344 rats (age not specified)	31M	30M (AAF alone)	sodium saccharin, 7 ppm *o*-toluenesulfonamide; method of production and purity not specified	0.02% AAF in diet for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Negative There was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma. There was a statistically significant increase in urothelial hyperplasia in the sodium saccharin-promoted rats.	Nakanishi et al. (1982)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.4 *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT)							
4-wk-old Fischer rats	1) 20M 2) 20M	3) 20M (sodium saccharin alone) 4) 20M (FANFT alone) 5) 42M (no exposure)	sodium saccharin (*o*-toluenesulfonamide free) Method of production and purity not specified	1) 0.2% FANFT for 6 wk followed by 5% sodium saccharin diet 2) 0.2% FANFT for 6 wk followed by 6-wk no-exposure period, followed by 5% sodium saccharin diet 3) 5% sodium saccharin diet alone 4) FANFT initiation alone 5) no exposure	83 wk	Positive with FANFT initiation Sodium saccharin was negative for bladder tumorigenesis when administered alone. Incidence of bladder cancer in groups 1, 2, 3, 4, and 5 were as follows: 18/19, 13/18, 0/20, 4/20, and 0/42, respectively.	Cohen et al. (1979)
5-wk-old F344 rats	26M	25M	sodium saccharin, method of production and purity not specified.	FANFT: 0.2% diet sodium saccharin: 5% diet	FANFT for 4 wk; sodium saccharin for 100 wk	Positive with FANFT pretreatment There was a significant increase ($p < 0.03$) in the incidence of carcinoma of the bladder as compared to FANFT-only controls (5/26 vs. 0/25).	Fukushima et al. (1981)
5-wk-old Fischer rats	20M	20M	sodium saccharin FANFT Method of production and purity not specified	5% diet 0.005% diet	2 yr	Equivocal The incidence of bladder lesions was marginally significant ($p < 0.06$), when compared to the incidence in FANFT-only controls (5/16 vs. 0/11). There were no statistically significant increases in tumor incidences for other tissues.	Murasaki and Cohen (1983a)
weanling F344 rats (age not specified)	42M (sodium saccharin and FANFT) 42M (sodium saccharin and DBN)	42M (FANFT alone) 43M (DBN alone) 42M (sodium saccharin alone)	sodium saccharin Method of production and purity not specified	4-wk exposure to either 0.2% FANFT in Wayne diet or 0.005% *N,N*-dibutylnitrosamine (DBN) in drinking water and control Wayne diet, followed by 5% sodium saccharin in AIN-76A diet for 100 wk	see dose	Negative None of the control rats fed sodium saccharin alone developed bladder, liver, or esophageal tumors. There was no statistically significant increase in the incidence of tumors of the bladder, liver, or esophagus in rats initiated with FANFT or DBN and subsequently promoted with sodium saccharin, as compared to FANFT-only and DBN-only controls, respectively. There was a non-statistically significant increase ($p=0.059$) in urinary bladder carcinomas in FANFT-initiated, sodium saccharin-promoted rats.	Imaida and Wang (1986)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	240M 160M 120M	40M[b] 40M[b] 40M[b] [b]shared controls	sodium saccharin calcium saccharin acid saccharin ascorbic acid sodium ascorbate	3 or 5% diet 3.12 or 5.2% diet 2.53% diet 4.44 % diet 5 % diet	72 wk	Positive with FANFT pretreatment. Without FANFT initiation, sodium saccharin, calcium saccharin, and acid saccharin were non-tumorigenic. With a 6 wk period of FANFT initiation, sodium saccharin was tumorigenic at the 5 and 3% dose levels. Calcium saccharin was slightly tumorigenic but not in a dose response manner. Acid saccharin was not tumorigenic. One of 2 diets was fed: Prolab 3200 or NIH-07. Urinary pH of rats fed Prolab was higher and sodium saccharin promoted more bladder tumors in these rats.	Cohen et al. (1991b)
5-wk-old F344 rats	240M 160M 120M	40M[b] 40M[b] 40M[b] [b]shared controls	sodium saccharin calcium saccharin acid saccharin ascorbic acid sodium ascorbate	3 or 5% diet 3.12 or 5.2% diet 2.53% diet 4.44 % diet 5 % diet	72 wk	Tumors were analyzed for H-*ras* mutations by Western blotting using a monoclonal antibody against p21. H-*ras* mutations were found in 2/3 and 3/6 bladder tumors from rats exposed to FANFT alone, and 4/20 and 1/10 H-*ras* mutations were found in tumors from rats exposed to FANFT initiation with 3 or 5 % sodium saccharin promotion, respectively.	Masui et al. (1991)
5-wk-old F344 rats	30M (FANFT + sodium saccharin)	30M (FANFT alone) 25M (sodium saccharin alone)	sodium saccharin, method of production and purity not specified	5% AIN-76A diet	100 wk	Negative Sodium saccharin did not promote bladder cancer in rats initiated for 4 wk with 0.2% FANFT and fed AIN-76A diet. This was probably due to the low urinary pH of rats fed AIN-76A diet.	Okamura et al. (1991)
5-wk-old F344 rats	30M (FANFT + sodium saccharin)	30M (FANFT alone)	sodium saccharin, method of production and purity not specified	5% Prolab diet	100 wk	Positive with FANFT pretreatment. Rats initiated with 0.2% FANFT for 4 wk and fed Prolab diet containing sodium saccharin had an increased incidence of bladder tumors, as compared to FANFT-controls (40% vs. 17% incidence of bladder tumors, respectively). Sodium saccharin was not administered alone in Prolab diet.	Okamura et al. (1991)
4.2.5 *N*-Methyl-*N*-nitrosourea (MNU)							
6- to 8-wk old Wistar rats	M and F (number used varied between reports)	M and F (number used varied between reports)	sodium saccharin, method of production and purity not specified	2 or 4 g/kg bw/day in diet or 2 g/kg bw/day in drinking water	2 yr	Positive with MNU pretreatment and dietary administration of sodium saccharin. Bladder tumor incidences were as follows: 3/138 in male and female 4 g/kg bw/day sodium saccharin-only group, 23/49 (47%) rats in the MNU plus 2 g/kg bw/day sodium saccharin female group, and 27/47 (57%) in the MNU plus 4 g/kg bw/day sodium saccharin female group. Administration of 2 g/kg bw/day of sodium saccharin in drinking water did not produce bladder tumors in either sex.	Hicks et al. (1973) preliminary report; Hicks et al., 1975); Hicks and Chowaniec (1977; cited by Whysner and Williams, 1996)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Wistar/AF-Han rats (age not specified)	F (number not specified), instilled with 2 g MNU in the bladder	F (number not specified)	sodium saccharin, method of production and purity not specified	2% sodium saccharin in the diet for the first 10 wk and 4% afterwards	≤ 2 yr	Negative for increase following MNU pretreatment In the MNU-only group, bladder tumors were found in 19/49 (39%) rats; and ureter tumors were found in 8/49 (17%) rats, while 14/49 (28%) rats developed renal pelvis tumors. In the MNU plus sodium saccharin group, incidences of renal pelvis, ureteran and bladder tumors were 43, 11 and 39% respectively. The high incidence of tumors in the MNU-only group was explained by the original authors as a result of the use of MNU within 15 min of dissolution.	Mohr et al. (1978)
Wistar rats (age not specified)	63F (MNU + sodium saccharin containing 40 mg/kg *o*-toluene-sulfonamid) 63F (MNU + sodium saccharin free of *o*-toluene-sulfonamid)	63F (MNU alone)	MNU sodium saccharin prepared by the Remsen-Fahlberg method, containing 40 mg/kg *o*-toluene-sulfonamide sodium saccharin prepared by the Maumee process, no *o*-toluenesulfonamide	0.15 mL instilled into bladder 2 g/kg/day in drinking water 2 g/kg/day in drinking water 2 g/kg/day in diet	single dose 2 yr (started 2 wk after MNU) 2 yr (started 2 wk after MNU) 2 yr (started 8 days after MNU)	Negative There was no increase in tumor incidence in rats administered *o*-toluene-sulfonamide-free sodium saccharin or in rats administered *o*-toluene-sulfonamide-free sodium saccharin, as compared to the MNU-only control group, but the latency period was shorter (55 and 52 wk vs. 87 wk for controls).	Hooson et al. (1980)
8-wk-old SD rats	960F 120F 120F	240F[b] 240F[b] 240F[b] [b] shared controls	sodium saccharin sodium saccharin acid saccharin Methods of production and purities not specified	0.1, 0.5, 1, 2.5, or 5% diet 2% drinking water 5% diet	102 wk	Positive with MNU pretreatment with dietary administration of sodium saccharin. Rats were given either a single dose (300 µL) of saline or an initiating dose (0.5 mg/300 µL saline) of MNU, a potent direct acting carcinogen, via trans-urethral instillation. A significant increase in the incidence of benign papillomas was seen in MNU-pretreated rats when fed 0.1-2.5% sodium saccharin. Rats which received 5% sodium saccharin had a benign papilloma incidence similar to controls. Acid saccharin also required MNU initiation for production of tumors. Sodium saccharin administered in drinking water was not as effective in producing tumors as was sodium saccharin administered in the diet.	West et al. (1986)
8-wk-old Sprague-Dawley rats	30F (saccharin alone) 60F (saccharin plus MNU)	78F	sodium saccharin, method of production and purity not specified	up to 5% given 4 wk before, during or after MNU initiation; rats then fed control diet	112 wk	Negative MNU-induced tumorigenesis was not enhanced by the 4-wk sodium saccharin exposure	West et al. (1994)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.6 Freeze Ulceration							
5-wk-old F344 rats	M (number not specified)	not specified	sodium saccharin, method of production and purity not specified	5%	102 wk	Positive with freeze ulceration pretreatment. In rats with freeze ulceration initiation followed by saccharin diet, 5/20 (25%) had carcinomas or papillomas. FANFT pretreatment for 2 wk after freeze ulceration and subsequent sodium saccharin exposure resulted in 4/23 (17%) incidences of bladder tumors. Reversing order of FANFT and freeze ulceration exposure resulted in an 8/22 (36%) incidence of bladder tumors. Tumors were not found in rats that received FANFT, sodium saccharin, or freeze ulceration alone.	Cohen et al. (1982)
5-wk-old F344 rats	40M per group (freeze ulceration followed 0, 2, 4, 6, or 18 wk later with sodium saccharin)	40M (sodium saccharin alone) 40M (freeze ulceration alone) 40M (no treatment)	sodium saccharin, method of production and purity not specified	5% diet	104 wk total (saccharin was administered either immediately after freeze ulceration or after 2, 4, 6, or 18 wk)	Positive with freeze ulceration pretreatment. There was a significant increase in the incidence of transitional cell carcinoma of the bladder in rats subjected to freeze ulceration and then fed sodium saccharin either immediately or 4, 6, or 18 wk later, as compared to freeze ulceration-only control (11/36, 6/36, 12/40, 7/36, 9/39 vs. 1/39 in controls). The increase was not significant in rats fed sodium saccharin 2 wks after freeze ulceration. None of the saccharin-only or no-treatment control rats developed bladder carcinoma.	Hasegawa et al. (1985)

Abbreviations: F = females; M = males

[a] No distinction was made between saccharin and its sodium salt in the IARC discussion

[b] No distinction was made between saccharin and its sodium salt

5.0 GENOTOXICITY

Extensive reviews of the genotoxicity of saccharin have been conducted by Ashby (1985), IARC (1980, pp. 148-150, see Appendix A; 1982, see Appendix B; 1987b, see Appendix C), and, most recently, by Whysner and Williams (1996). The studies summarized below are largely based on these reviews; additional, relevant studies are presented in **Table 5-1**, while the Genetic Activity Profiles published by IARC (1987a) for saccharin are provided in **Figures 5-1** and **5-2.**

Most of the numerous *in vitro* and *in vivo* genotoxicity studies on sodium saccharin have been negative with occasional inconsistent or conflicting results and false positive results attributed to factors such as mutagenic impurities, inhibition of DNA synthesis, and osmotic effects.

5.1 Noneukaryotic Systems

5.1.1 Gene Mutations

Both sodium saccharin and saccharin (form unspecified) have been reported as negative in 15 studies for the induction of reverse mutations in *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538 (not all strains were tested in all studies), with and without S9 activation, and using either the plate incorporation or pre-incubation forms of the assay.

In a study that evaluated the induction of reverse mutations in *S. typhimurium* by 4 commercially available saccharin samples and by 1 highly purified saccharin sample in the presence and absence of S9, the commercially produced samples were positive for mutation induction, whereas the highly purified sample was negative (Batzinger et al., 1977). The author concluded that commercial saccharin samples contain mutagenic impurities.

5.1.2 DNA Damage

Saccharin (form unspecified) was reported as negative for the induction of prophage (Rossman et al., 1991) and DNA damage/SOS repair in *Escherichia coli* (DeFlora et al., 1984).

5.1.3 DNA Synthesis

Saccharin did not alter DNA synthesis, as measured by the incorporation of [^{3}H]thymidine, in *S. typhimurium* (Beljanski et al., 1982).

5.2 Lower Eukaryotic Systems

5.2.1 *Saccharomyces cerevisiae*

Sodium saccharin without metabolic activation was reported to be positive in the yeast, *Saccharomyces cerevisiae*, for the induction of aneuploidy, gene conversion, and reverse mutations. However, in two other yeast studies (including one conducted using a 9-fold higher dose), saccharin (form unspecified) was negative for gene conversion and mutation induction but positive again for the induction of aneuploidy.

5.2.2 *Drosophila melanogaster*

Sodium saccharin was initially found in a 1971 study to be positive for the induction of sex-linked recessive mutants and negative for heritable translocations in *Drosophila*

melanogaster. However, two subsequent studies conducted using equal or higher doses reported weak positive or negative results for the induction of sex-linked recessive mutations.

5.2.3 Higher Plants

Ma et al. (1995) concluded that saccharin induced micronuclei in the root-tips of *Allium cepa* (onions) and *Vicia fava* (beans) following a 6-hour exposure at 120 mM.

5.3 Mammalian Systems *In Vitro*

5.3.1 Gene Mutations

In two studies, sodium saccharin was reported to be weakly mutagenic in mouse lymphoma L5178Y cells at very high doses (>10 mg/mL) and only in the presence of metabolic activation. A third study using doses as high as 20 mg/mL up was negative. Sodium saccharin, at doses above 10 mg/mL, was also reported to induce a highly significant increase in the number of ouabain-resistant mutants in human RSa embryo cells (Suzuki and Suzuki, 1988), and to increase the number of mutations at the k-*ras* gene, codon 12 in SW480 human colon adenocarcinoma cells (Suzuki and Suzuki, 1993). However, based on either the weakness of the response and/or the magnitude of the doses required to elicit a positive response, these data would be considered to be of questionable value using current testing practices.

5.3.2 DNA Damage

Sodium saccharin (without metabolic activation) was weakly positive or positive for the induction of sister chromatid exchanges (SCE) in three studies using Chinese hamster cells and in two studies using human lymphocytes. Sodium saccharin and saccharin, in the absence of metabolic activation, were reported to be negative for SCE induction in one study using mouse embryo fibroblasts and in two studies using human lymphocytes. Studies with metabolic activation were either not conducted or were negative for SCE induction. In the positive studies, the doses capable of inducing a significant increase in SCE ranged from 1 to 12 mg/mL and the maximum increase in SCE was generally less than two-fold. As discussed by Ashby (1985) and based on our current appreciation of the various processes involved in SCE induction, this increase in SCE more likely reflects the ability of saccharin at high doses to inhibit DNA synthesis rather than an ability to cause DNA damage.

5.3.3 Inhibition of DNA Repair

Skare and Wong (1985) reported that sodium saccharin did not inhibit the repair of UV-induced DNA damage in WI-38 human fetal lung fibroblasts.

5.3.4 DNA Synthesis

Yanagisawa et al. (1987) and Heil and Reifferscheid (1992) both concluded that sodium saccharin at relatively high doses inhibited the rate of DNA synthesis, as measured by incorporation of [^{3}H]thymidine after treatment, in human B-32 fibroblasts or HeLa S3 cells.

5.3.5 Chromosomal Damage

Sodium saccharin was found to be positive without S9 activation in ten studies and negative in two studies for the induction of chromosome aberrations using Chinese hamster cells and human lymphocytes. Ashby (1985) and Whysner and Williams (1996) concluded that the high dose levels used (up to 48 mg/mL) may have caused osmotic changes leading to false positive results.

5.3.6 Cell Transformation

Saccharin (form unspecified) was found to give negative results for cell transformation in BALB/c3T3 and C3H 10T1/2 mouse and RLV Fischer rat embryo cells. Sakai and Sato (1989) also reported that sodium saccharin did not increase the number of transformed foci in BALB/3T3 cells with or without a 2-week promotion period with TPA.

5.4 Mammalian Systems *In Vivo*

5.4.1 Gene Mutations and Dominant Lethal Mutations

In a study that compared the mutagenic activities of 3 commercially available saccharin samples with a highly purified saccharin sample, Batzinger et al. (1977) administered 2.5 g saccharin/kg to mice (strain not specified) and collected 24-hour urine samples. The urine samples were then assayed for mutagenicity in *S. typhimurium* strains TA98 and TA100 in the presence and absence of S9 and the enzyme β-glucuronidase. In strain TA98, all commercial samples were positive for the induction of reverse mutations, but the purified sample was negative. In strain TA100, all samples were positive. Mutagenic activities of the urine was enhanced in TA98 by β-glucuronidase. In TA100, mutagens were inactivated by S9; in TA98, mutagens were activated by S9. The authors proposed that 2 mutagenic substances were present. A similar study using TA 98, TA 100 and TA 1537 performed on urine obtained from rats treated with 5% dietary sodium saccharin, Hasegawa et al. (1984) failed to show mutagenic activity after 0, 1, 5, or 14 days of treatment.

Batzinger et al. (1977) also conducted a host-mediated assay for the induction of reverse mutations by the 4 saccharin samples (3 commercially available, 1 highly purified). *S. typhimurium* strain TA98 or TA100 was incubated for 6 hours in the peritoneal cavity of mice administered 2.5 g saccharin/kg. The highly purified sample was negative for mutation induction in both bacterial strains. All of the commercially available samples were positive, except for one sample that was negative when incubated with strain TA98.

Both negative and positive results were obtained for sodium saccharin in the mouse spot test, examining somatic cell mutations induced in pup coat color. In his review of the literature, Ashby (1985) reported that the difference may have been due to the different routes of exposure (i.p. vs. orally, respectively) and the 7.5-fold higher oral dose levels in the positive study.

As compiled by Ashby (1985), IARC (1987a,b) and Whysner and Williams (1996) and discussed by Adler and Ashby (1989), saccharin has given conflicting results in the mouse dominant lethal mutation assay, with three positive and three negative studies for sodium saccharin. The strain of mice and the route of exposure were often the same and the doses often

overlapped for both negative and positive studies. The authors of the review articles concluded that the *in vivo* mutagenic ability of saccharin has not been adequately demonstrated.

5.4.2 DNA Damage

Sodium saccharin, when administered orally at doses between 5 and 10 g/kg, was reported to induce up to a two-fold increase in SCE in Chinese hamster bone marrow cells (Renner, 1979). Dropkin et al. (1985) reported that sodium saccharin at doses up to 25 mg/kg/day did not cause sister chromatid exchanges in the fetal pups of female ICR albino mice dosed on the 10th day of gestation and sacrificed on the 17th day.

5.4.3 Chromosomal Aberrations

In the reviews conducted by Ashby (1985), IARC (1987a,b), and Whysner and Williams (1996), both sodium saccharin and saccharin (form unspecified) were reported as negative for the induction of chromosomal damage in somatic and germ cells of rodents in seven studies and positive in somatic and germ cells in one study each. Dropkin et al. (1985) also reported that sodium saccharin at doses up to 2000 mg/kg did not cause chromosome aberrations in the fetal pups of female ICR albino mice dosed on the 10th day of gestation and sacrificed on the 17th day.

5.4.4 Induction of Micronuclei

Sodium saccharin was reported in two studies as negative for micronucleus induction in mouse bone marrow cells.

Table 5-1. Summary of Saccharin Genotoxicity Studies

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryotic Systems							
5.1.1 Gene Mutations							
Salmonella typhimurium strains TA98 and TA100	Induction of reverse mutations	+/-	saccharin (4 commercially available samples and 1 highly purified sample); n.p.	No; up to 40 mg/plate (commercially available samples) or up to 80 mg/plate (highly purified sample); incubation time not specified	positive (commercially available samples) negative (highly purified sample)	There was considerable variation in mutagenic activity among the 4 commercially available samples.	Batzinger et al. (1977)
S. typhimurium strains TA98, TA100, and TA1537	Induction of reverse mutations	+/-	5% dietary sodium saccharin, method of production and purity not specified.	Yes; 0. 0.2 and 0.3 mL urine used on days 0, 1, 5, and 14 of treatment.	negative (commercially available samples)	No dose-response relationship was observed. Results were interpreted as no mutagens being present in the urine following freeze-ulceration and/or sodium saccharin feeding.	Hasegawa et al. (1984)
5.1.2 DNA Damage							
Escherichia coli strain WP2	Lambda prophage induction (microscreen assay)	+/-	saccharin, n.p.	No; 100 µg/well for 20 h	negative/ negative	No enhancement of plaque forming units per plate	Rossman et al. (1991)
5.1.3 DNA Synthesis							
S. typhimurium	Stimulation of DNA synthesis	-	saccharin, n.p.	Yes; 1, 10, 20, 30, and 40 µg/ assay for 10 min	negative	Measured [3H]thymidine DNA synthesis	Beljanski et al. (1982)
5.2 Lower Eukaryotic Systems							
5.2.3 Higher Plants							
Allium cepa (onion) and *Vicia faba* (broad bean)	Micronucleus assay	-	saccharin, n.p.	Yes; 40, 80, and 120 mM for 6 h followed by 44 h recovery	positive	Significant increase in micronuclei 80 and 120 mM	Ma et al. (1995)

Table 5-1. Summary of Saccharin Genotoxicity Studies (Continued)

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.3 Mammalian Systems *In Vitro*							
Human RSa embryo cells; SW480 human colon adenocarcinoma cells	Mutations at k-ras codon 12	-	sodium saccharin, n.p.	Yes; 10 to 30 mg/mL for 24 h	positive	DNA was extracted, amplified by PCR, dot-blotted, and hybridized to labeled probes, positive at 15 mg/mL	Suzuki and Suzuki (1993)
5.3.1 Gene Mutations							
Human RSa embryo cells	Mutations to ouabain resistance	-	sodium saccharin, n.p.	Yes; 10 to 22.5 mg/mL for 24 h	positive	Dose dependent increase in mutant frequency with top dose being 45-fold higher than controls	Suzuki and Suzuki (1988)
5.3.3 Inhibition of DNA Repair							
WI-38 human fetal lung fibroblasts	Inhibition of DNA repair synthesis	-	sodium saccharin, n.p.	Yes; 10, 57, 319, 1785, and 10,000 µg/mL for 4 h	negative	Measured incorporation of [3H]thymidine after UV irradiation	Skare and Wong (1985)
5.3.4 DNA Synthesis							
Human B-32 fibroblasts	DNA synthesis inhibition	+	saccharin sodium, n.p.	No; 0.1 M for 0, 30, or 90 min	positive	Measured [3H]thymidine incorporation following treatment	Yanagisawa et al. (1987)
HeLa S3 cells	DNA synthesis inhibition	-	saccharin, n.p.	No; DI50 (concn. which inhibited DNA synthesis by 50%) - 140 mM for 90 min	positive	Measured incorporation of BrdU using anti-BrdU antibody	Heil and Reifferscheid (1992)
5.3.6 Cell Transformation							
BALB/3T3 cells	Morphological cell transformation	-	sodium saccharin, n.p.	No; dose not provided, 72 h treatment followed by 2 wk with or without TPA promotion	negative	No increase in transformed foci with or without TPA	Sakai and Sato (1989)

Table 5-1. Summary of Saccharin Genotoxicity Studies (Continued)

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.4 Mammalian Systems *In Vivo*							
5.4.1 Gene Mutations and Dominant Lethal Mutations							
mice (strain not specified)	Induction of reverse mutations	+/-	saccharin (3 commercially available samples and 1 highly purified sample); n.p.	No; 2.5 g/kg by gavage	TA98: positive (all commercial samples)/ negative (purified sample) TA100: positive (all samples)	The mutagenic activities of 24-hour urine samples were assayed in *S. typhimurium* strains TA98 and TA100. The strains were incubated both in the presence and absence of β-glucuronidase. Mutagenic activities of the urines were enhanced in TA98 by β-glucuronidase. In TA100, mutagens were inactivated by S9; in TA98, mutagens were activated by S9. The authors proposed that 2 mutagenic substances were present.	Batzinger et al. (1977)
mice (strain not specified)	Induction of reverse mutations	-	saccharin (3 commercially available samples and 1 highly purified sample); n.p.	No; 2.5 g/kg by gavage	TA98: positive (all commercial samples)/ negative (purified sample) TA100: positive (2/3 commercial samples)/ negative (1/3 commercial samples; purified sample)	*S. typhimurium* strain TA98 or TA100 was incubated for 6 hours in the peritoneal cavity of mice administered saccharin.	Batzinger et al. (1977)
5.4.2 DNA Damage							
ICR albino mice (pregnant)	SCE	-	saccharin sodium, >99%	Yes; i.p., 5, 10, and 25 mg/kg/day	negative	Dams dosed on 10th day of gestation and sacrificed on day 17	Dropkin et al. (1985)
5.4.3 Chromosomal Aberrations							
ICR albino mice (pregnant)	Chromosomal aberrations	-	saccharin sodium, >99%	Yes; i.p., 1000 and 2000 mg/kg	negative	Dams dosed on 10th day of gestation and sacrificed on day 17	Dropkin et al. (1985)

Figure 5-1. Genetic Activity Profile (GAP) for Saccharin

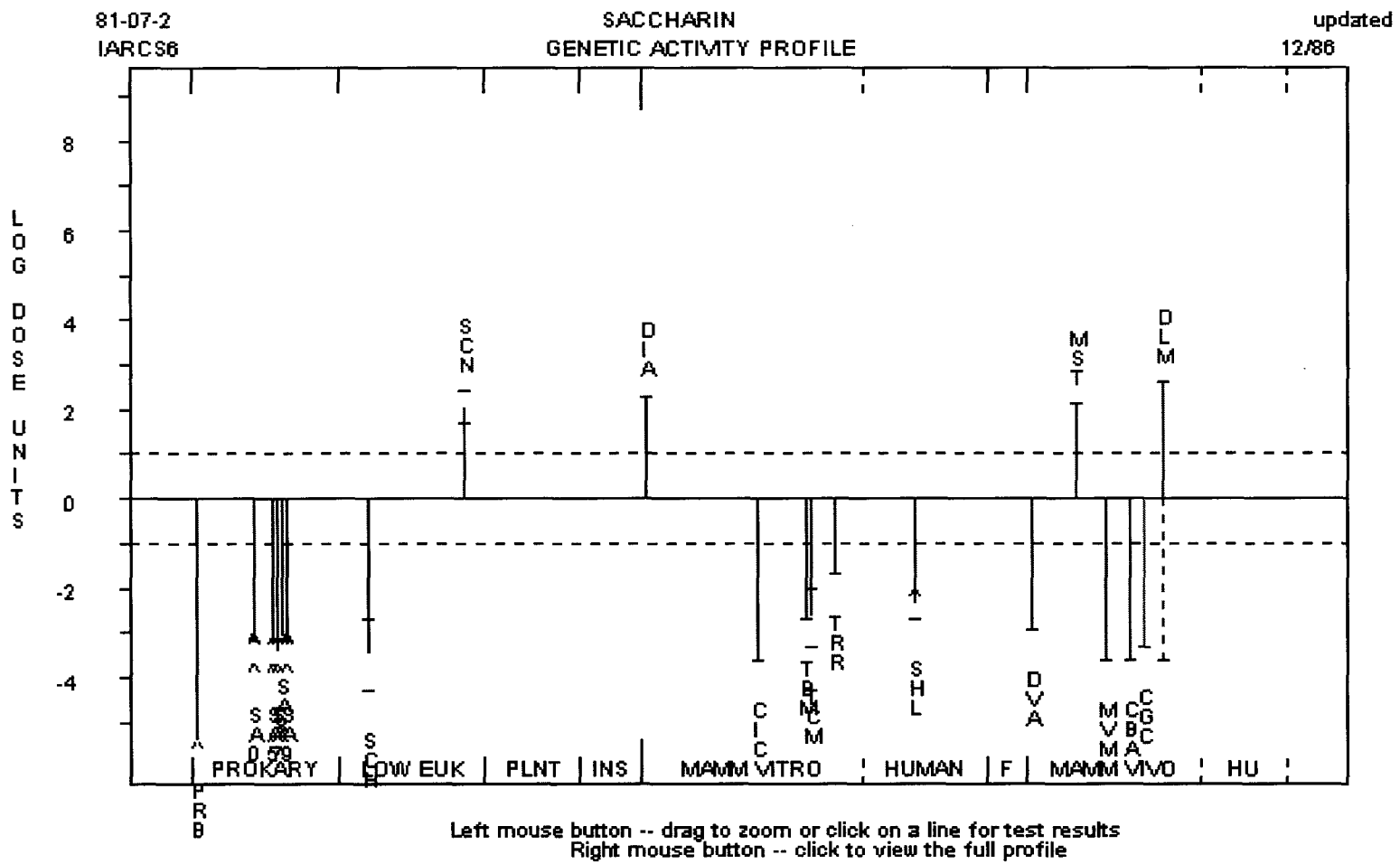


Figure 5-2. Genetic Activity Profile (GAP) for Sodium Saccharin

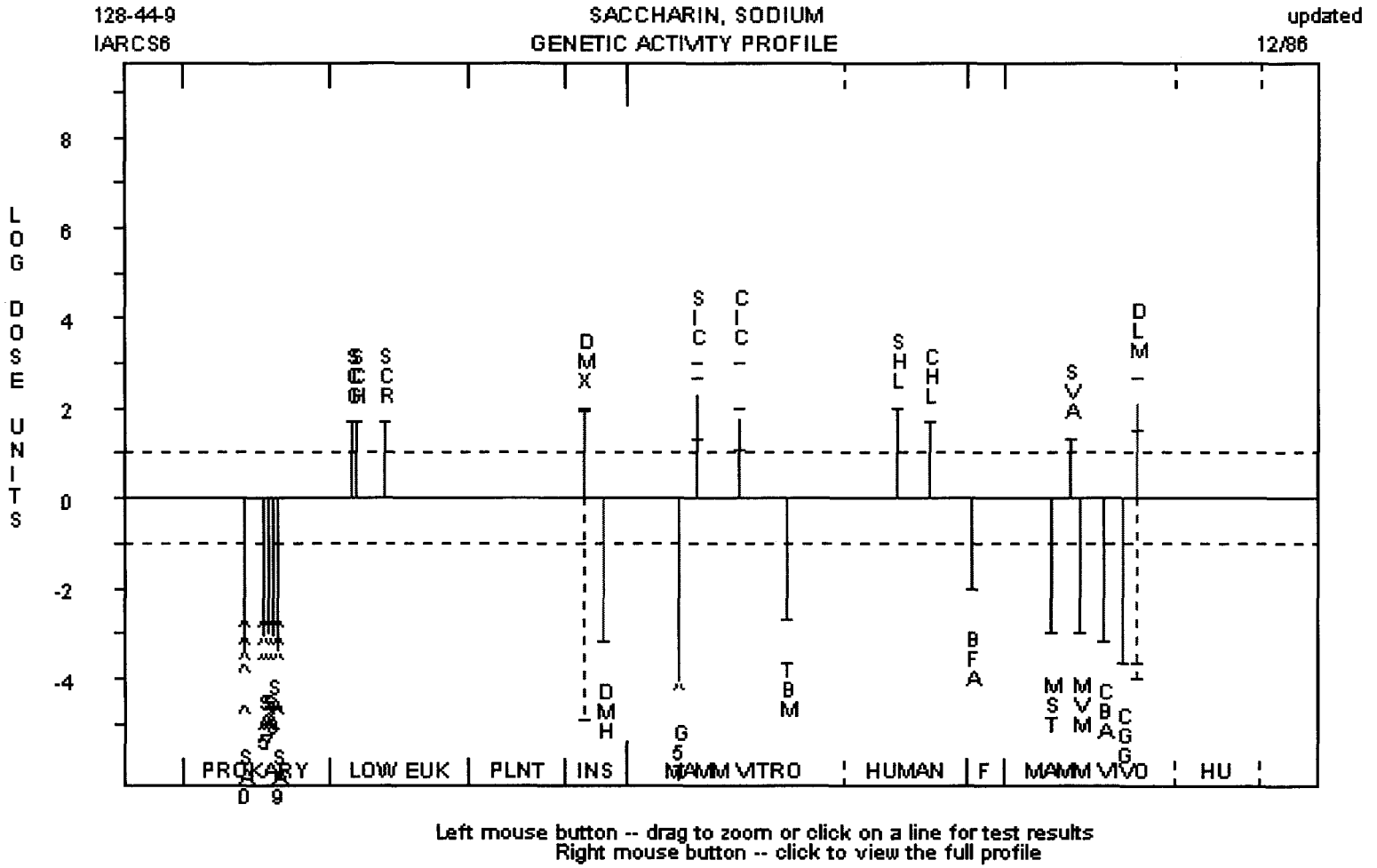
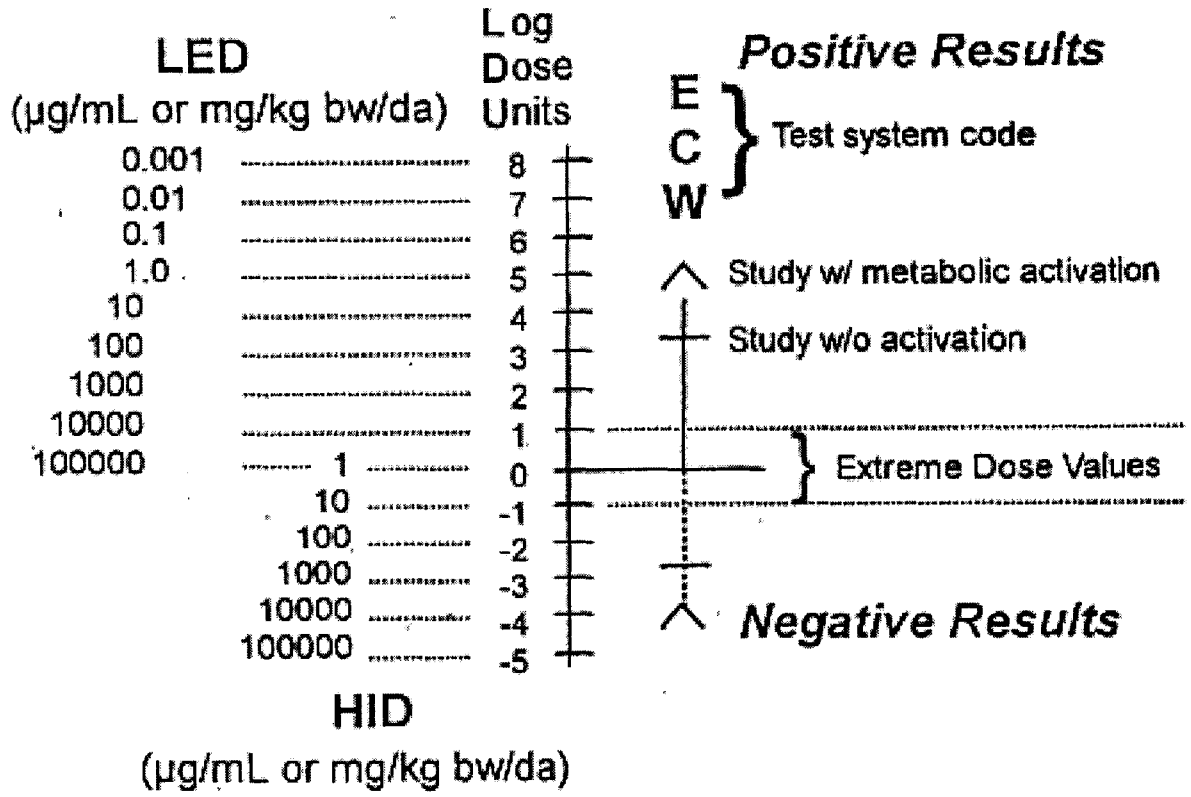


Figure 5-3. Schematic View of a Genetic Activity Profile



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N. E., H. F. Stack, M. R. Gross, and M. D. Waters. 1984. An Analysis of the Spectra of Genetic Activity Produced by Known or Suspected Human Carcinogens. Mutat. Res. 134:89-111.

Waters, M. D., H. F. Stack, A. L. Brady, P. H. M. Lohman, L. Haroun, and H. Vainio. 1988. Use of Computerized Data Listings and Activity Profiles of Genetic and Related Effects in the Review of 195 Compounds. Mutat. Res. 205:295-312.

Waters, M. D., H. F. Stack, N. E. Garrett, and M. A. Jackson. 1991. The Genetic Activity Profile Database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT DATA

Summary: Saccharin is a polar synthetic compound that is not a substrate for normal intermediary metabolism and is not used as an energy source. Earlier metabolic investigations using radiolabeled techniques indicated that saccharin underwent limited metabolism by ring opening to 2-sulfamoylbenzoic and 2-sulfabenzoic acids. However, these findings were not confirmed in later more extensive studies conducted on humans and rats using similar radiolabeled techniques. In humans, saccharin is almost completely (90%) absorbed from the intestinal tract and excreted unchanged in the urine largely (90%) by renal tubular secretion within 24 hours after oral administration. Human data fitted a two compartment model (plasma and renal clearance, half-life [$t_{1/2}$] about 70 minutes) for intravenous (i.v.) administration of a bolus dose of sodium saccharin dihydrate ($NaSac \cdot 2H_2O$).

After rats were i.v. dosed with [5-3H]saccharin, the plasma concentration-time curve clearly showed a biphasic decline during the first 2 hours, and about 90% of the dose was recovered in urine which was found to be consistent with the elimination $t_{1/2}$ (30 minutes). At low doses (100 mg/kg or less) the plasma clearance (about 10 mL min^{-1} kg^{-1}) decreased at high doses (1000 mg/kg) to 5.5 mL min^{-1} kg^{-1}, with the recovery in urine in 2 hours decreasing to 65% of the dose. The elimination $t_{1/2}$ (30 minutes) was found to be similar for all doses less than 1000 mg/kg.

With occasional exceptions, studies in male and female rats dosed with 5% or greater levels of sodium saccharin in the diet typically show alterations in the ultrastructural morphology of urinary bladder urothelium, enhanced proliferation as evidenced by elevated labeling indexes (LIs), and morphological evidence of urothelial hyperplasia. These effects can be seen as soon as 90 days after commencement of in utero treatment and generally within 10 wk when treatment starts shortly after weaning, especially when treatment is preceded or accompanied by treatment with a urinary bladder initiator. It has been shown that the severity of urothelial changes is influenced by diet. Urinary bladder changes have been demonstrated in male and female rats but not in other species tested.

6.1 Absorption, Distribution, and Excretion

Sweatman et al. (1981) dosed three adult human males ages 25 to 37 years with saccharin either orally (2 g in gelatin capsules after an overnight fast or 1 to 2 hours after breaking fast) or i.v. (sodium saccharin dihydrate, 10 mg/kg) and recorded the excretion of saccharin over 96 hours. The results indicated that saccharin was almost completely (90%) absorbed from the intestinal tract after oral administration and excreted unchanged in the urine largely by renal tubular excretion, mostly within the first 24 hours of dosing. This study also found that saccharin administered either orally or intravenously resulted in 90% recovery of the dose in the urine and up to 8% in the feces.

In studies it was found that saccharin does not accumulate in any tissues, including the bladder (Renwick, 1986). Sweatman and Renwick (1980) studied eighteen adult male and six adult female rats fed ad libitum a diet containing 1 to 10%, and 5%, respectively, sodium saccharin dihydrate for 22 days. High-pressure liquid chromatography was used to detect the concentration of saccharin in tissues (well perfused; poorly perfused) and plasma. Saccharin

underwent significant plasma protein binding (69 to 86%) at all dietary levels. The well perfused tissues (adrenal, liver, lung, and spleen) contained 20 to 50% lower concentrations of saccharin than the corresponding plasma concentrations at each dietary level. The lowest levels of saccharin detected (10 to 20% plasma level) were found in poorly perfused tissues (muscle and fat). The highest concentrations were found in the gut wall. A tissue-to-plasma ratio greater than unity was observed in the kidneys (101.6 μg/mL : 29.6 μg/mL [3.43]) and urinary bladder (120.7 μg/mL : 29.6 μg/mL [4.1]). Although the tissue distribution was similar between male and female rats fed a diet containing 5% saccharin, the tissue concentrations of saccharin were higher in females than those found in males (liver, 2.4-fold; lung, 3-fold; muscle, 2.6-fold; kidney, 1.5-fold; bladder, 6.7-fold).

Sweatman and Renwick (1982) studied whether or not a two-generation feeding protocol was associated with uniquely elevated concentrations of saccharin in the bladder or other tissues of rats. Following a single oral dose of [3H]saccharin (sodium saccharin dihydrate; 50 mg/kg; 1.0 to 3.0 mCi; >99.8% pure) to female Sprague-Dawley rats in late pregnancy, concentrations of 3H in tissues of dams at 6 to 12 hr after administration of the dose were higher than those of the fetuses. At 6 hr, maternal liver, kidney, and bladder wall concentrations were ~5-fold, ~33.3-fold and ~16.7-fold, respectively, higher than those of the fetuses. At 12 hr, maternal liver, kidney, and bladder wall concentrations were ~1.4-fold, ~8.3-fold, and ~5.8-fold, respectively, higher than those of fetuses. The concentrations of 3H in fetal tissues decreased more slowly at 48 hr, exceeding the corresponding values obtained for maternal tissues: liver, 3.2-fold; kidney, 0.8-fold, and bladder wall, 5.4-fold. The authors suggested that these findings point to the possible accumulation of saccharin during chronic intake (Sweatman and Renwick, 1982).

In another experiment conducted by Sweatman and Renwick (1982), dams were fed a 5% saccharin diet ad libitum from 4 wk prior to mating until killed during late gestation. The observed liver and kidney concentrations were lower in the fetuses than the corresponding maternal values: liver, 80 μg/g maternal vs. 36.5 μg/g fetal; kidney, 382 μg/g maternal vs. 198 μg/g fetal. However, the average concentration of saccharin in fetal bladder tissue was approximately 3.8-fold higher than the corresponding maternal value. The saccharin levels in the bladder, but no other tissue, of females (189 ± 149 μg/g) were significantly lower than in males (292 ± 261 μg/g) ($p < 0.05$ by unpaired Student's t-test). Between days 17 and 20, the concentration of saccharin in the amniotic fluid increased (males [n = 5], 15 μg/g; females [n = 7], 20 μg/g to males [n = 12] 361 μg/g; females [n = 18] 276 μg/g), which is a similar finding to Ball et al. (1977) who stated that the increase was possibly due to elimination of saccharin in fetal urine.

Liver concentrations of saccharin in F_1 animals exposed to a 5% saccharin diet reached a maximum of approximately 50 μg/g soon after weaning (between days 28 and 45). Due to the variability in the levels of saccharin in the bladder, no distinct maximum concentration was observed in F_1 animals. In previous studies conducted by Matthews et al. (1973), Lethco and Wallace (1975), Ball et al. (1977), and Sweatman and Renwick (1980), variability in the concentration of saccharin in the bladder wall was reported, either after a single dose or after chronic administration. Statistical analysis of the total bladder wall data showed that female levels were significantly (50%; $p < 0.05$) lower than males when each individual result was expressed as a percentage of the mean for the animals killed at that time point (to eliminate temporal variation). Between days 18 and 23, which corresponds to the time of separation from

the mother and consumption of a 5% saccharin diet, the average concentration of saccharin in urine of F_1 animals showed a marked increase (males, [n = 5] 4.6 μg/mL vs. [n = 2] 17.9 μg/mL; females, [n = 2] 8.6 μg/mL vs. [n = 5] 11.1 μg/mL) (Sweatman and Renwick, 1982).

Sweatman and Renwick (1982) studied the distribution and turnover of [3H]saccharin in pregnant rats maintained on a 5% saccharin diet prior to mating and transferred to a 5% saccharin diet radiolabeled with [3H] (6.1 μCi/g) on the 10th day of gestation. On days 10 to 20 of gestation, the concentrations of [3H]saccharin in maternal and fetal livers were similar to the unlabeled concentrations found by HPLC on day 20 (see above), indicating that steady-state concentrations had been reached. In the fetal tissues, the levels of [3H]saccharin showed a relatively uniform distribution. However, markedly lower concentrations were found in the brain. Similar findings were reported by Ball et al. (1977). The concentrations of [3H]saccharin were below the limit of detection in the fetal bladder. Sweatman and Renwick (1982) suggested that this was due to the size of the fetal bladder and the relatively low specific activity of the [3H]saccharin diet given. There was a marked reduction in the 3H concentrations in most maternal and fetal tissues upon transferring back to an unlabeled 5% saccharin diet for 24 hr or 48 hr prior to killing. Tritium concentrations in fetal liver, kidney, and muscle decreased to an average of 29, 45, and 22%, respectively, of the steady-state level after 24 hr on the unlabeled saccharin diet, while the corresponding maternal tissues decreased to 19, 51, and 23%, respectively. Tritium concentrations were not detectable (< 200 μg/g) in the fetal bladder wall throughout the duration of [3H]saccharin diet (10-20 days).

Ball et al. (1977) studied three groups of rats, one on a normal diet without pretreatment with [^{14}C]sodium saccharin for up to 12 months, and the others pre-treated with 1% or 5% saccharin diet for up to 12 months. Individual rats in each group were subsequently administered an oral dose of 16 to 22 mg/kg (5 to 9 μCi) sodium saccharin. In both groups about 95% of the dose was eliminated within 24 hours, with 72 to 92% detected in the urine and 0 to 22% detected in the feces. Within 3 days of dosing, excretion of ^{14}C was essentially complete. The final recovery in 6 days averaged 100%, with the urine containing 77 to 97% and feces containing 6 to 22% of the labeled dose. Pre-treatment of rats with a diet containing 1% and 5% saccharin for up to 12 months did not alter the pattern of absorption and excretion. The only alteration of this pattern was increased concentrations of [^{14}C]saccharin in the feces after continued intake, especially at the 1% dietary level. The authors also investigated the excretion of [^{14}C]saccharin in urine after i.p. injection and very little was associated with the gastrointestinal tract. The authors concluded that the increased concentration of [^{14}C]saccharin in feces after oral administration arose from incomplete absorption in the gut.

Lethco and Wallace (1975) administered [3-^{14}C]saccharin (5, 50, and 500 mg/kg) to male and female rats. The distribution of radioactivity in organs and tissues at various time intervals was monitored. One hour after administration of a 50 mg/kg dose, traces of ^{14}C were found in almost all of the organs. Saccharin reached a peak blood concentration within 8 hours. The kidney, urinary bladder, and liver tissues contained the highest ^{14}C concentration. All of the tissues except brain and spleen contained traces of ^{14}C 72 hours after dosing. The rats excreted 66 to 84 % of the labeled dose of [3-^{14}C]saccharin in the urine and 10 to 40% in the feces. This study also compared the metabolic profiles of a dog, rabbit, guinea pig, and hamster. When

compared, the metabolic profiles indicated that there was very little difference in the pattern due to dose level or animal species.

The absorption, distribution, and excretion of radiolabeled saccharin was studied by Matthews et al. (1973). Male rats (seven groups of three or more) were studied after receiving a single oral dose of [^{14}C]saccharin (1 mg/kg in 0.5 cm3 distilled water). The dose was administered orally to animals that had been fed ad libitum or fasted overnight. Saccharin entered the bloodstream rapidly, most likely due to absorption through the stomach, with peak concentrations in the blood between 7.5 and 15 minutes after administration. Saccharin was absorbed by the fasted animals more rapidly than those that were fed. The saccharin concentration of fasted animals was approximately twice that found in animals fed ad libitum. The time to peak saccharin concentration and the general shape of the curve were similar in the kidney and blood. The authors found that glomerular filtration of saccharin from the blood and its excretion in the urine resulted in temporary accumulation of 5 times more saccharin in the kidneys than in other organs or tissues. Saccharin was detected in the urine taken from the bladders of every rat as soon as 3 minutes post saccharin administration.

The accumulation and clearance of multiple doses of saccharin was also investigated by Matthews et al. (1973). Saccharin (1 mg/kg/day) was administered to two groups of four rats each for 7 days. Saccharin concentrations in the major organs were measured 24 and 72 hours after administering the final dose. At 24 hours, the saccharin concentration was slightly higher in the gastrointestinal tract and considerably higher in the bladder than in any other tissues. The authors suggested that although elevated concentrations of saccharin were not present in these tissues, the tissues may have absorbed saccharin from their contents rather than by distribution of the blood. Most of the saccharin had been cleared from all of the tissues by 72 hours after the last dose, with none of the tissues having a significantly higher concentration than the others at that time. The authors also stated that the ratio of saccharin excreted in the urine and feces was approximately 9:1 when analyzed during the feeding period and after the last dose of saccharin had been administered.

The authors continued this study by treating rats 5 times with a dose of 1 mg/kg at 90-minute intervals for a total dose of 5 mg/kg within a 6-hour period. This dosing regimen was used to simulate the daily dose of saccharin humans would be expected to consume be using saccharin in food or beverages several times throughout the day. The rats receiving multiple doses of saccharin were reported to have a higher saccharin concentration in tissues than in the corresponding tissues of rats which had received single doses of saccharin (1 mg/kg). Rats that were sacrificed 90 minutes after the fifth (last) 1 mg/kg dose were found to have a saccharin concentration in the kidneys equal to or greater than 5 times the concentration of kidneys from rats which received only a single dose of 1 mg/kg. Thereafter, the saccharin concentration in the kidneys of all of these rats approached 9 to 10 times that of the animals which received only a single dose. Twenty-four hours later, the difference had decreased to approximately 2-fold. A 10-fold difference was observed after 24 hours between concentrations in the bladders of rats receiving multiple doses and those of rats receiving single doses. Still, at 24 hours, the concentration in the bladders of rats which received 5 doses was less than 10% of that observed 90 minutes after the last of the 5 doses (Matthews et al., 1973). These data showed that

significant concentrations of saccharin can occur in certain tissues such as the kidney and bladder that appear to be almost completely cleared by the following day.

6.2 Metabolism

Saccharin is a polar synthetic compound that is not a substrate for normal intermediary metabolism and is not used as an energy source (Renwick, 1986). Metabolic investigations using radiolabeling techniques have indicated that saccharin undergoes limited metabolism by ring opening to 2-sulfamoylbenzoic and 2-sulfobenzoic acids (Pitkin et al., 1971; Kennedy et al., 1972; Arnold, 1983; Renwick, 1986). Kennedy et al. (1972) fed [^{14}C]saccharin to two rats (1 male and 1 female). Components of solvent extracts from their acidified urine were separated by thin layer chromatography (TLC) and compared to the authentic 2-sulfamoylbenzoic and sulfobenzoic acids. This experiment showed that in the urine samples collected between 0 to 24 hours after dosing, 0.4% to 0.6% of the dose was excreted as 2-sulfamoylbenzoic acid and less than 0.1% to 0.6% of the dose as 2-sulfobenzoic acid.

Pitkin et al. (1971) studied the metabolism of [^{14}C]saccharin in eight female Rhesus monkeys using the same method as Kennedy et al. (1972), which was unpublished at the time. The authors reported that [^{14}C]saccharin was excreted essentially unchanged in the urine of monkeys. The authors also found that urine samples collected and analyzed between 24 to 48 hours and 48 to 72 hours after dosing contained 1.2% of the dose as 2-sulfamoylbenzoic acid and 0.1% as 2-sulfobenzoic acid.

The Food and Drug Administration also detected 2-sulfamoylbenzoic in a more extensive study that focused on the metabolic profiles of a dog, guinea pig, hamster, rabbit, and six rats exposed to [3-^{14}C]saccharin via gavage (Lethco and Wallace, 1975). In this study six rats (three males and three females) were given oral doses of 5, 50, and 500 mg/kg [3-^{14}C]saccharin (10 to 15 μCi/kg). Twenty-four hours after the dose was administered, the rate of $^{14}CO_2$ expiration, and [^{14}C]carbonate and 2-sulfamoylbenzoic acid excreted via the urine were identified using paper chromatography, TLC, UV spectrophotometry, and reverse isotope dilution techniques. These data showed that both male and female rats expired $^{14}CO_2$ between 0.5 and 8 hours after dosing, while only female rats expired 0.01% of the dose at 24 hours. Male rats expired a total of 0.29, 0.03, and 0.10% of 5, 50, and 500 mg/kg doses, respectively, and female rats expired a total of 0.23, 0.55, and 0.27% of the 5, 50, and 500 mg/kg, doses, respectively, 24 hours post-dose. When 24 rats (12 males and 12 females; 4 rats/dose) were dosed with 5, 50, and 500 mg/kg [3-^{14}C]saccharin, about 0.4% of the dose was excreted as 2-sulfamoylbenzoic acid in the urine with approximately equal amounts identified as ^{14}C carbonate as detected by DEAE cellulose chromatography (Lethco and Wallace, 1975; Renwick, 1986). Generally, more than 99% of the urinary radioactivity was unmetabolized saccharin and all of the species' urine samples contained small amounts of 2-sulfamoylbenzoic acid. Comparative metabolic profiles of a dog, rabbit, guinea pig, and hamster indicated that there was little difference in the pattern due to animal species or dose level (Lethco and Wallace, 1975). The authors suggested that the breakdown of saccharin was due to a chemical reaction as opposed to enzymatic reactions.

Ball et al. (1977) used chromatographic, reverse isotope dilution techniques, and UV spectrophotometric techniques for the detection of radiolabeled metabolites of saccharin (2-sulfamoylbenzoic acid and $^{14}CO_3^{2-}$ in the urine, $^{14}CO_2$ in expired air). The limits of detection

were as low as 0.03% for $^{14}CO_2$ and $^{14}CO_3^{2-}$. Rats were fed a diet containing 1% or 5% of saccharin for up to 12 months prior to receiving a [^{14}C]saccharin dose (20 mg/kg) administered orally. The authors were unable to detect any metabolism in either the urine or in the expired air of the rats dosed with radiolabeled saccharin. Ball et al. (1977) were also unable to detect any metabolites of saccharin in the urine of three adult humans (one female, two males; 55 to 94 kg body weight) who ingested 1 g saccharin/day for 22 days as a treatment prior to receiving a final dose of [3-^{14}C]-saccharin (20 μCi; 13 mg) on the 22nd day. The authors were also unable to detect metabolite production from the three adult humans when they were not subjected to the saccharin pretreatment before a dose of [3-^{14}C]saccharin (20 μCi/13 mg).

Sweatman and Renwick (1979) exposed male rats to saccharin both in utero and during lactation. The authors were unable to detect any metabolites of saccharin in the excreta of rats under these conditions. The authors also reported that after 3-methylcholanthrene treatment (inducer of metabolism), saccharin metabolites were undetectable using reverse isotope dilution with limits of detection as low as 0.01% for 2-sulfamoylbenzoic acid. These results found that significant metabolism is not induced by long term administration of saccharin during the neonatal and weaning-stages of two generations.

Clearly, a discrepancy between some of the earlier reports and later investigations exist. Earlier studies may have used saccharin with slight impurities resulting in metabolism of the impure substance. Pitkin et al. (1971) used benzene ring-labeled [^{14}C]saccharin from Mallinckrodt Chemical Corp. Byard and Golberg (1973) reported that the benzene ring-labeled [^{14}C]saccharin supplied by Mallinckrodt Chemical Works (St. Louis, MO, USA) contained an impurity which produced a 2 to 3% metabolic reaction if given to animals. In brief, the authors found that the metabolite produced *in vivo* from the impurity chromatographed as 2-sulfamoylbenzoic acid but did not recrystallize with added 2-sulfamoylbenzoic acid. Both Matthews et al. (1973) and Byard and Golberg (1973) found that solvent extraction and t.l.c. in neutral solutions would give rise to artifactual metabolites. In neither the Kennedy et al. (1972) study which used [3-^{14}C]saccharin from Monsanto Co. (St. Louis, MO, USA), nor the Pitkin et al. (1971) study, which used [^{14}C]saccharin from Mallinckrodt Chemical Corp, was the purity of the saccharin specified. It seems likely that the results obtained from experiments conducted by Kennedy et al. (1972) and Pitkin et al. (1971) might be due to some unidentified impurity similar to that found by Byard and Golberg (1973). Experiments aimed at the induction of metabolism of [^{14}C]saccharin by pretreatment with phenobarbital (Byard and Golberg, 1973) also failed to induce metabolic reactions producing 2-sulfamoylbenzoic acid, 2-sulfobenzoic acid, CO_2, or the carbonate.

Lethco and Wallace (1975) explained the presence of [^{14}C]saccharin metabolites as a slight breakdown of saccharin due to simple decomposition rather than enzymatic mechanisms. Although the authors' data were substantiated by the large number of animals studied and the consistency of the extent of metabolism over a wide range of doses in various species, the saccharin molecule is resistant to chemical decarboxylation and thus slight breakdown to CO_2 and CO_3^{2-} seems unlikely (Renwick, 1986).

6.3 Pharmacokinetics

The human data generated by Sweatman et al. (1981) fitted a two-compartment model (plasma and renal clearance) for i.v. administration of a bolus dose of saccharin (sodium saccharin dihydrate; 10 mg/kg) in the presence or absence of probenecid (competes for and inhibits renal tubular secretion of organic ions). Probenecid was administered (500 mg) 2 and 12 hours before and 2 hours after the i.v. dose of saccharin. Saccharin was rapidly eliminated via the urine after i.v. administration ($t_{1/2}$ about 70 min). A significant decrease in the elimination rate constant (40%) and in the plasma clearance (36%) rate occurred when the i.v. dose was given during probenecid treatment. Thus, tubular secretion is responsible for the elimination of a minimum of 40% of circulating saccharin in humans, which is consistent with the high renal clearance noted in this study. The fact that plasma clearance values were slightly less than the corresponding renal clearance suggests the absence of significant metabolism. This supports earlier studies using [^{14}C] saccharin in humans (Byard et al., 1974; Ball et al., 1977) that failed to detect significant metabolism after oral administration.

Sweatman and Renwick (1980) dosed ten male rats with low i.v. bolus doses (1, 20, 50 mg/kg). The plasma concentration-time curve clearly showed a biphasic decline during the first 2 hours. The plasma levels fit the equation

$$C_p = 3.12De + 1.35De^{-0.0213t},$$

where C_p is the plasma concentration in $\mu g\ mL^{-1}$ at time (t) and D is the dose in mg/kg. About 90% of the dose was recovered in the urine within 2 hours. This finding is consistent with the elimination $t_{1/2}$ (30 minutes).

6.4 Structure-Activity Relationships

No data were found.

6.5 Cell Proliferation

Experimental details for the studies described in this section are presented in **Table 6-1**.

6.5.1 Hamsters

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male Syrian hamsters administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.2 Mice

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male B6C3F1 mice administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.3 Rats

Lessel (1971) reported that saccharin was positive for hyperplasia in rats exposed to a 5% saccharin diet for 2 yr. Of 5 bladders from animals exposed to 5% saccharin, 1 male and 1 female had urothelial hyperplasia. IARC (1980) noted the small number of bladders examined histologically.

A 5% sodium saccharin diet fed to 6-wk-old F344 male rats for up to 18 wk induced vacuolar degeneration of the bladder urothelium after 3 wk and simple hyperplasia at 5 wk. The degree of hyperplasia increased with a display of mitotic figures, hyperplastic foci and pleomorphic microvilli starting at 9 wk. Increased thymidine uptake (5- to 8-fold the rate seen in controls) was present in the bladders of exposed rats at all time periods measured through 18 wk (Fukushima and Cohen, 1980).

Hooson et al. (1980) reported mild focal urothelial hyperplasia in 1/50 female Wistar rats exposed to 2 g sodium saccharin/kg/day for 2 yr. IARC (1980) noted that the rats were not started on the test diet at weaning, but after several wk on a normal diet.

Six-week-old male and female F344 rats fed up to 5% sodium saccharin in a stock diet alone for 32 wk did not develop simple, papillary, or nodular hyperplasia (Nakanishi et al., 1980a). However, rats initiated with BBN for 4 wk and then fed 5% sodium saccharin stock diet for 32 wk developed papillary and nodular hyperplasia (Nakanishi et al., 1980a).

In a 2-generation study, male and female F_1 Charles River CD rats exposed to up to 7.5% sodium saccharin in the diet for up to 2 yr had an increased incidence of urinary bladder hyperplasia at the 7.5% dose, but it was not morphologically precancerous. Exposure to 0.01, 0.1, 1.0, or 5.0% sodium saccharin had no effect on the incidence of hyperplasia (Taylor et al., 1980).

Lawson and Hertzog (1981) reported that sodium saccharin did not induce DNA synthesis in male Sprague Dawley rat bladder epithelium, as measured by an LI or by specific activity of DNA. Animals were fed 7.5% sodium saccharin diet for 50 wk with interim sacrifices throughout. [Methyl-^{3}H]thymidine was injected intraperitoneally 1 hour before death.

Murasaki and Cohen (1981) studied the dose response relationship between sodium saccharin exposure and cell proliferation in the urinary bladders of five-week-old male F344 rats fed sodium saccharin in the diet for 10 wk. The results of this experiment showed a dose-related increase in tritiated thymidine LI, the presence of pleomorphic microvilli, and hyperplasia. The no-observable-effect-level (NOEL) for statistically significant changes in LI was 0.1%.

The incidences of simple hyperplasia (25/32 vs. 1/28 controls) and papillary or nodular hyperplasia (20/32 vs. 0/28 controls) were significantly increased in male ACI rats administered 5% sodium saccharin in the diet for 52 wk beginning at 6 wk of age. At least half of the ACI rats were infected with the bladder parasite *Trichosomoides crassicauda* (Fukushima et al., 1983). Females were not evaluated.

Male F344 rats fed a 5% sodium saccharin diet for up to 20 wk beginning at 6 wk of age developed hyperplasia of the urinary bladder and significantly increased DNA synthesis at 20 wk (Fukushima et al., 1983).

Sodium saccharin induced hyperplasia of the urinary bladder in male ACI rats but not in F344, Sprague Dawley, or Wistar rats administered 5% sodium saccharin in the diet for 52 wk beginning at 6 wk of age. However, the concentration of urinary $MgNH_4PO_4$ crystals was greater in all strains of treated rats than in their respective controls (Fukushima et al., 1983). The ACI rats also developed urinary bladder papillomas and carcinomas. Females were not evaluated.

The effects of sodium saccharin on freeze ulceration-induced cell proliferation in male F344 rats were studied by Murasaki and Cohen (1983b). The authors found that the degree of microvilli formation and hyperplasia was similar for the 2-wk period following freeze ulceration

whether or not 5% sodium saccharin was administered immediately following the procedure. In another experiment, Murasaki and Cohen (1983b) found that sodium saccharin administered 2 or 8 wk following freeze ulceration produced a similar increase in hyperplasia, LI, and microvilli.

Hasegawa and Cohen (1986) studied the impact of the cation associated with different dosage forms of saccharin. In male F344 rats fed a 5% sodium saccharin, acid saccharin, potassium saccharin, or calcium saccharin diet for 10 wk, the LI was increased approximately 2-fold for calcium saccharin, 3-fold for potassium saccharin, and 9-fold for sodium saccharin. No increased LI was found for acid saccharin, and only the increased LI associated with sodium and potassium salt exposures reached statistical significance. A statistically significant increase in rats with hyperplasia was found in the sodium saccharin-treated group. Evidence of simple hyperplasia following potassium saccharin and calcium saccharin exposure, and increases in microvilli with potassium saccharin exposure were found. However, these changes were not statistically significant.

Tatematsu et al. (1986) found that a 5% sodium saccharin diet for 21 wk did not increase DNA synthesis in the bladder epithelium of male Fischer rats.

A 2-generation study conducted by Masui et al. (1988 abstr.), evaluated the urinary bladder proliferative effects upon fetal and neonatal Sprague-Dawley rats of both sexes, when their dams were fed a 5% sodium saccharin diet prior to mating and up to weaning. In control and sodium saccharin-treated fetuses at days 17 and 21 of gestation, the LIs were similar in both groups. Similar LIs were also found for both exposed and control rats at day 7 after birth. However, the LI was greater in sodium saccharin-treated rats (higher in females than in males), at day 21 after birth, compared to controls.

Garland et al. (1989b) found that the proliferative effects of sodium saccharin were dependent upon diet. In Experiment 1, five-week-old male F344 rats were given 5 or 7.5% sodium saccharin in Prolab 3200, NIH-07 or AIN-76 diet for 4 or 10 wk. In Experiment 2, male F344 rats and 4-wk-old male Sprague-Dawley rats were dosed with 5 and 7.5% sodium saccharin in Prolab 3200 or Purina 5002 diet for 10 wk. The results of Experiment 1 showed that sodium saccharin had a greater effect on bladder urothelium in the rats fed the Prolab diet compared with those on the NIH diet. In addition, there was little effect in the rats on the AIN diet. Effects included urothelial hyperplasia at 4 and 10 wk and an increased thymidine LI for the Prolab and NIH diet at 10 wk. In Experiment 2, the response was greater in F344 rats than in Sprague-Dawley rats and greater for the Prolab rather than the Purina diet for hyperplasia, increased LI, and evidence of urothelial damage.

Male F344 rats were exposed to 3, 5, or 7.5% sodium saccharin diet (Prolab feed) for 4, 7, or 10 wk in a dose-response experiment conducted by Cohen et al. (1990). Cell exfoliation and necrosis were evident at 10 wk in the group fed 3% sodium saccharin. An apparent progression from mild to more severe necrotic changes during the 4- to 10-wk period was found in the 5 and 7.5% sodium saccharin group. In the 5% sodium saccharin-exposed group, a doubling of the LI with extensive cell damage was noted. In the 7.5% sodium saccharin-exposed group, the LI was increased several fold, with evidence of hyperplasia.

The effects of diet on cell proliferation induced by sodium saccharin were also studied by Debiec-Rychter and Wang (1990). Male F344 rats were exposed to 5% sodium saccharin in either Wayne or AIN-76A diet for 2, 4, 6, 10, or 16 wk. Both diets increased the LI

approximately 5-fold when measured at 2, 4, 6, 10, or 16 wk. The authors also found that 2% sodium bicarbonate increased the LI for the AIN-76A diet 6- to 9-fold. In addition, a sodium saccharin and sodium bicarbonate combination proved to have an additive effect on cell proliferation, except at the 2-wk interval. A similar study was not conducted for the Wayne diet.

Garland et al. (1991) reported that sodium saccharin at 7.5% dietary concentration was positive for hyperplasia in male SD rats exposed *in utero* from conception up to 90 days of age. Urothelial hyperplasia was not present at 30 days of age.

Two separate studies conducted by Garland et al. (1994) and Uwagawa et al. (1994) demonstrated that NCI-Black-Reiter (NBR) rats, which do not produce 2-globulin (the male rat-specific, low molecular weight urinary protein), do not exhibit sodium saccharin-induced urinary bladder cell proliferation. Male NBR, F344, and castrated F344 rats were fed 7.5% sodium saccharin in Prolab 3200 diet for 10 wk. The most severe changes were found in both normal and castrated sodium saccharin-exposed F344 rats. Hyperplastic changes were found in the bladders of 7/10 intact F344 rats compared with 1/10 NBR rats. Hyperplasia was not found in the bladders of control rats. Although the 2μ-globulin urinary content in castrated F344 rats has been reported to be only 10% of that in intact normal F344 rats (Roy and Neuhaus, 1967; cited by Garland et al., 1994), examination of the saccharin-treated castrated F344 rats urinary bladders revealed that 4/10 showed signs of hyperplasia (Garland et al.,1994).

Uwagawa et al. (1994) exposed 6-wk-old F344 and NBR rats to 5% sodium saccharin, 5% sodium ascorbate, or 3% uracil for 8 wk. In both strains, the most severe urothelial changes were induced by uracil as shown by scanning electron microscopy (SEM). Sodium ascorbate-induced simple hyperplasia was found in the bladders of F344 rats but not in NBR rats. Sodium saccharin did not induce hyperplasia in the bladders of NBR; uracil-induced hyperplasia, however, was found in both strains. Increases in the BrdU LIs were found in F344 rats administered uracil (> 50-fold), ascorbate (36-fold), or sodium saccharin (20-fold).

Fischer 344 rats exposed to a 7.5% sodium saccharin diet for 10 wk developed hyperplasia. Amorphous precipitate was present in exposed rats along with an increased incidence of urothelial simple hyperplasia (Cohen et al., 1995a).

Sodium saccharin was positive for cell proliferation in male and female F344 rats exposed to a 5% sodium saccharin diet for 21 or 91 days; the effects were reversible over time (Cohen et al., 1995b).

6.5.4 Guinea Pigs

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male Hartley guinea pigs administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.5 Nonhuman Primates

Sodium saccharin was negative for cell proliferation in Macaca mulatta monkeys fed 20, 100, or 500 mg/kg/day in diet for 79 months. Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology (McChesney et al., 1977 abstr.; cited by IARC, 1980).

6.6 Cell Proliferation with Co-Administration of Known Carcinogens

6.6.1 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)

Urinary bladder hyperplasia was enhanced in 6-wk-old male and female F344 rats exposed to 2000, 10,000, or 50,000, but not 400 ppm, sodium saccharin in the diet following BBN pretreatment. Exposure to sodium saccharin without BBN pretreatment did not induce any changes in urinary bladders of rats of either sex (Nakanishi et al., 1980a).

The effects of sequential administration (initiation/promotion protocol) of 0.01% BBN in drinking water and 5.0% sodium saccharin in feed and concurrent administration of 0.001% BBN in drinking water and 5.0% sodium saccharin in feed, were studied in 8-wk-old male Wistar rats by Nakanishi et al. (1980b). In the first experiment (sequential administration), rats received BBN for 4 wk and then sodium saccharin for an additional 32 wk. In the second experiment (concurrent administration), rats were fed both BBN and sodium saccharin for 40 wk. There was an enhancement of urinary bladder hyperplasia and bladder tumors when rats were exposed to BBN and sodium saccharin either sequentially or concurrently, while sodium saccharin alone caused urinary bladder urothelial hyperplasia.

Nakanishi et al. (1982) reported that there was a significant increase ($p < 0.05$) in the incidences of simple, papillary, or nodular urinary bladder hyperplasia in male F344 rats (age not specified) initiated with 0.01% BBN in drinking water for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone (simple hyperplasia: 27/29 vs. 19/28; papillary or nodular hyperplasia: 24/29 vs. 11/28).

6.6.2 2-Acetylaminofluorene (AAF)

Sodium saccharin was positive for hyperplasia in female Horton SD rats fed a 5% sodium saccharin diet for 40 wk with co-administration of AAF. Hyperplasia of the urinary bladder mucosal lining occurred in all control and treated females fed AAF. The hyperplasia was most pronounced in the AAF/sodium saccharin-exposed animals, with one of these rats displaying squamous metaplasia and precancerous changes in the mucosal epithelium. IARC noted that the small number of animals used, and the fact that food consumption was not measured, prevented the evaluation of AAF and sodium saccharin exposure (Ershoff and Baja, 1974; cited by IARC, 1980).

Nakanishi et al. (1982) reported that there was a significant increase ($p < 0.05$) in the incidences of simple, papillary, or nodular urinary bladder hyperplasia in male F344 rats (age not specified) initiated with 0.02% AAF in the diet for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone (simple hyperplasia: 6/29 vs. 0/28; papillary or nodular hyperplasia: 4/29 vs. 0/28).

6.6.3 *N*-Methyl-*N*-nitrosourea (MNU)

There was an increase in the number of proliferative bladder lesions in female Wistar rats (age not specified) administered a single 0.15 mL intravesicular dose of MNU, followed 2 wk later by daily administration of either 2 g/kg *o*-toluenesulfonamide-free sodium saccharin or 2 g/kg sodium saccharin containing 40 mg/kg *o*-toluenesulfonamide for 2 yr, as compared to a control group given MNU alone (incidence not given) (Hooson et al., 1980).

Table 6-1. Cell Proliferation

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6.5.1 Hamsters							
6-wk-old Syrian golden hamsters	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluenesulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.2 Mice							
6-wk-old B6C3F1 mice	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluenesulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.3 Rats							
Boots-Wistar rats (age not specified)	40M, 40F	20M, 20F	saccharin[a], made by Remsen-Fahlberg method, purity not specified	0.005, 0.05, or 5% diet	2 yr	Positive with highest dose Of 5 bladders from animals exposed to the highest dose, 1 male and 1 female had urothelial hyperplasia. IARC (1980) noted the small number of bladders examined histologically.	Lessel (1971)
6-wk-old Charles River F344 rats	24M	6M	sodium saccharin, methods of production and purity not specified	5% diet	<18 wk	Positive Three treated rats were killed at 1, 3, 5, 7, 9, 12, 15, and 18 wk. Three controls killed at 0 and 18 wks. Vacuolar degeneration of the epithelial cells at 3 wk and simple hyperplasia at 5 wk were observed. At 9 wk, the degree of hyperplasia increased with occurrences of mitotic figures, hyperplastic foci and pleomorphic microvilli. Thymidine LIs were increased in bladders of exposed rats at all time periods measured.	Fukushima and Cohen (1980)
Wistar rats (age not specified)	50F	63F	sodium saccharin, made by Maumee process, purity not specified	2 g/kg body weight/day	2 yr	Negative Mild focal urothelial hyperplasia was seen in one rat fed sodium saccharin. IARC (1980) noted that animals were started on the test diet not at weaning, but after several wk on a normal diet.	Hooson et al. (1980)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old inbred Charles River F344 rats	302M, 311F	29M, 30F	sodium saccharin, methods of production and purity not specified	0.04, 0.2, 1, or 5% diet with or without 4 wk of BBN pretreatment	32 wk	Negative Sodium saccharin alond did not induce simple, papillary or nodular hyperplasia except after pretreatment with BBN in the 5% groups of males and females.	Nakanishi et al. (1980a)
in utero Charles River CD rats	240M, 240F	8M, 48F	sodium saccharin, made by Remsen-Fahlberg method, 350 ppm *o*-toluenesulfonamide	0.01, 0.1, 1, 5, or 7.5% diet	2 yr	Positive with highest dose This was a 2-generation study. Males and females exposed to 7.5% sodium saccharin had an increased incidence of urinary bladder hyperplasia, but it was not morphologically precancerous. Exposure to 0.01, 0.1, 1, or 5% sodium saccharin had no effect on the incidence of hyperplasia.	Taylor et al. (1980)
3-wk-old Sprague-Dawley CD weanling rats	labeling index (LI) measurement group: 8M sacrificed at 3 defined durations of treatment specific activity of DNA measurement group: varying numbers (19-24M) sacrificed at 9 defined durations of treatment	LI measurement group: 8M sacrificed at 3 defined durations of treatment specific activity of DNA measurement group: varying numbers (19-23M) sacrificed at 9 defined durations of treatment	sodium saccharin incorporated in the diet and then pelleted, purity not specified	7.5% sodium saccharin diet plus [Methyl-^{3}H]thymidine injected intraperitoneally 1 h before death	LI measurement group: 1, 15, and 50 wk specific activity of DNA measurement group: 1, 2, 3, 6, 10, 15, 20, 30, and 50 wk	Negative Sodium saccharin did not increase bladder epitheleal DNA synthesis (measured by the LI and by specific activity of DNA).	Lawson and Hertzog (1981)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old Fischer F344 rats	10M (for each dose level)	10M	sodium saccharin, made by Maumee process, purity not specified	0.1, 0.5, 1, 2.5, 5% diet	10 wk	Positive above 0.1% Sodium saccharin did not induce formation of papillary or nodular hyperplasia, papilloma, or cancer. The LI increased significantly in a dose response manner at dose above 0.1%. Administration of 1, 2.5, or 5% sodium saccharin increased the number of foci containing ropy microridges or uniform microvilli in intestines.	Murasaki and Cohen (1981)
rats (strain and age not specified)	M (number not specified)	M (number not specified)	sodium saccharin, methods of production and purity not specified.	5% diet	10 wk	Positive Dose-related increase in tritiated thymidine LI and the presence of uniform and pleomorphic microvilli and hyperplasia were observed. The no-observable-effect-level (NOEL) for statistically significant changes in LI was 0.1%.	Murasaki and Cohen (1981)
6-wk-old ACI rats	48M	45M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Positive The incidences of urinary bladder simple hyperplasia (25/32 vs. 1/28 controls) and papillary or nodular hyperplasia (20/32 vs. 0/28 controls) were significantly increased. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*.	Fukushima et al. (1983)
6-wk-old F344 rats	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	up to 20 wk	Positive Sodium saccharin induced hyperplasia of the urinary bladder and significantly increased DNA synthesis at 20 wk.	Fukushima et al. (1983)
6-wk-old F344 rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder, but the concentration of $MgNH_4PO_4$ crystals in the urine of treated rats was greater than that in controls.	Fukushima et al. (1983)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old SD rats and Wistar rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder, but the concentration of $MgNH_4PO_4$ crystals in the urine of treated rats was greater than that in controls.	Fukushima et al. (1983)
5-wk-old inbred Fischer 344 rats	5-13M sacrificed at 9 defined durations up to 8 wk	5-13M sacrificed at defined durations up to 8 wk: 4 sacrifice dates for group receiving freeze ulceration + control diet and 7 sacrifice dates for groups receiving either sodium saccharin or control diet alone	sodium saccharin mixed in the diet and pelleted, purity not specified	5% diet either immediately after or 2 wk after freeze ulceration	8 wk	Positive Nodular and papillary hyperplasia and luminal surface abnormalities were detected when rats were fed sodium saccharin either immediately after freeze ulceration or 2 wk after freeze ulceration. Incidences high for entire 8 wk of the experiment.	Murasaki and Cohen (1983b)
5-wk-old inbred Fischer 344 rats	M (number not specified)	M (number not specified)	sodium saccharin mixed in the diet and pelleted, purity not specified	5% diet administered 8 wk after freeze ulceration 5% diet administered 2 wk after freeze ulceration	16 wk	Positive Development of nodular and papillary lesions, surface abnormalities, and increased LI were similar to results reported in the two groups above.	Murasaki and Cohen (1983b)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	M (number not specified)	M (number not specified)	sodium saccharin acid saccharin potassium saccharin calcium saccharin Methods of production and purity not specified	5% diet 5% diet 5% diet 5% diet	10 wk	Positive Sodium saccharin induced significant urinary bladder epithelial proliferation. Potassium saccharin also did, but not as much. Calcium saccharin and acid saccharin did not induce a significant increase in proliferation.	Hasegawa and Cohen (1986)
Fischer rats (age not specified)	not specified	not specified	sodium saccharin, methods of production and purity not specified	5% diet	21 wk	Negative Exposure did not increase DNA synthesis in the bladder epithelium.	Tatematsu et al. (1986)
fetal and neonatal Sprague-Dawley rats	not specified	not specified	sodium saccharin, methods of production and purity not specified	5% diet fed to dams before mating until weaning	fed to dams before mating until weaning	Positive At day 21 after birth, the LI in bladder was greater for exposed rats than control rats. The LI was higher in exposed females than in exposed males.	Masui et al. (1988 abstr.)
5-wk-old F344 rats and 4-wk-old Sprague-Dawley rats	105M	60M	sodium saccharin, 99.9% pure; method of production not specified	5 or 7.5% diet	4 or 10 wk	Positive when diet made urine alkaline One of 3 diets was fed: Prolab 3200, NIH-07, or AIN-76A. There was a higher incidence of simple or nodular hyperplasia of urothelium in rats fed Prolab than those fed NIH diet. There was little response with AIN diet. Urinary pH in rats fed AIN diet was 6.0 ± 0.0. Rats fed NIH diet had a urinary pH of 6.3 ± 0.2 and rats fed Prolab had a urinary pH of 6.4 ± 0.2. The response to sodium saccharin was greater in F344 rats than SD rats.	Garland et al. (1989b)
28-day-old F344 rats	30M (for each dose level)	30M	sodium saccharin, methods of production and purity not specified	3, 5, or 7.5% diet	4, 7, or 10 wk	Positive with highest dose Light microscopic changes in bladder and an increase in LI in bladder were seen at all time points but only in rats fed 7.5% dose level. Scanning electron microscopic changes were seen beginning at 4 wk, with increasing severity at higher doses.	Cohen et al. (1990)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Weanling F344 rats (age not specified)	18-20M per group	20M (AIN-76A), M (number not specified; Wayne diet)	sodium saccharin, methods of production and purity not specified	5% diet AIN-76A or Wayne diet	2,4,6,10, or 16 wk	Positive Sodium saccharin in both diets caused a significant increase in the thymidine LI. Sodium bicarbonate alone increased the LI and in combination with sodium saccharin had an additive effect on bladder urothelial LI. A sodium bicarbonate study was not done with the Wayne diets.	Debied-Rychter and Wang (1990)
in utero and 30-day-old Sprague-Dawley rats	7M, 7F for each dose level (*in utero*); 7M, 7F for each dose level (30-day-old)	7M, 7F (*in utero*); 7M, 7F (30-day-old)	sodium saccharin, 99.2% pure; method of production not specified	1, 3, or 7.5% diet	30, 60, or 90 days	Positive with highest dose *In utero* rats were exposed to sodium saccharin from conception to 30 days. Thirty-day-old rats were exposed for 60 days. Mild simple hyperplasia of the urinary bladder occurred in 90-day-old male rats (4 cases) fed 7.5% sodium saccharin, one 30-day-old female rat fed 7.5% sodium saccharin, and eight 90-day-old female rats fed 7.5% sodium saccharin. There were 2 cases of moderate or severe hyperplasia in 90-day-old female rats fed 7.5% sodium saccharin and 1 case in a 30-day-old female control rat. One 30-day-old female control rat exhibited moderate or severe hyperplasia. Significance values were not included.	Garland et al. (1991)
4- to 5-wk-old intact F344, castrated F344, and NBR rats	10M (intact), 10M (castrated), 10M (NBR)	10M (intact), 10M (castrated), 10M (NBR)	sodium saccharin, 98.1% pure with no impurities > 1 ppm; method of production not specified	7.5% diet	10 wk	Positive only in rats that synthesized α2μ-globulin NBR rats don't synthesize α2μ-globulin. Castrated rats have lower levels than intact rats. Sodium saccharin produced less bladder proliferation in NBR rats than in intact F344 rats. Intermediate proliferation was seen in castrated rats.	Garland et al. (1994)
6-wk-old NBR and F344 rats	6M (NBR), 6M (F344)	10M (NBR), 5M (F344)	sodium saccharin, methods of production and purity not specified	5% diet	8 wk	Positive only in rats that synthesized α2μ-globulin NBR rats do not synthesize α2μ-globulin. Only F344 rats had an increase in cell proliferation in urinary bladder after exposure to sodium saccharin.	Uwagawa et al. (1994)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	10M	10M	sodium saccharin, pure, method of production not specified	7.5% diet	10 wk	Positive Amorphous precipitate was present in exposed rats along with an increased incidence of urothelial simple hyperplasia.	Cohen et al. (1995a)
8-wk-old and 6-wk-old F344 and Sprague-Dawley rats	M, F (numbers not specified)	not specified	sodium saccharin acid saccharin Methods of production and purity not specified	5% diet 5% diet	21 or 91 days	Positive (sodium saccharin) Sodium saccharin and acid saccharin were evaluated. Neither increased bladder proliferation when fed at birth through 7 days of age. Sodium saccharin increased proliferation at later times but acid saccharin did not. The effects of sodium saccharin were reversible over time.	Cohen et al. (1995b)
6.5.4 Guinea Pigs							
6-wk old Hartley guinea pigs	30M	20M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.5 Nonhuman Primates							
Macaca mulatta monkeys (age not specified)	7M, 7F	3M, 3F	sodium saccharin, made by Remsen-Fahlberg method, containing 2.4 or 3.2 mg/kg *o*-toluene-sulfonamide	20, 100, or 500 mg/kg bw/day in diet	79 mo	Negative Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology.	McChesney et al. (1977 abstr.; cited by IARC, 1980)
6.6 Cell Proliferation with Co-Administration of Known Carcinogens							
6.6.1 *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)							
6-wk-old F344 rats	242M, 249F	60M, 62F	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	400, 2000, 10,000, or 50,000 ppm diet with or without BBN pretreatment	32 wk	Positive with higher doses and BBN pretreatment Urinary bladder hyperplasia was enhanced in both sexes by exposure to 2000-50,000 ppm sodium saccharin following BBN pretreatment. Exposure to sodium saccharin without BBN pretreatment did not produce any changes in urinary bladders of rats of either sex.	Nakanishi et al. (1980a)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
8-wk-old Wistar rats	40M (BBN/sodium saccharin)	36M (BBN alone), 32M (sodium saccharin alone), 18M (no chemicals)	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]	sodium saccharin: 5% diet; 0.01% in drinking water	Rats pretreated with BBN for 4 wk and then given sodium saccharin for 32 wk	Positive with BBN treatment There was an enhancement of urinary bladder papillary or nodular hyperplasia (21/31 vs. 6/23 BBN controls).	Nakanishi et al. (1980b)
8-wk-old Wistar rats	40M (BBN/sodium saccharin)	24M (BBN alone), 24M (sodium saccharin alone), 18M (no chemicals)	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]	sodium saccharin: 5% diet BBN; 0.001% in drinking water	Rats were co-administered BBN and sodium saccharin for 40 wk	Positive with BBN treatment There was an enhancement of urinary bladder hyperplasia (simple hyperplasia, 24/24 vs. 2/22 BBN controls; papillary or nodular hyperplasia, 20/24 vs. 2/22).	Nakanishi et al. (1980b)
F344 rats (age not specified)	31M	30M (BBN alone)	Sodium saccharin [7 ppm *o*-toluenesulfonamide]; methods of production and purity not specified	0.01% BBN in drinking water for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Positive with BBN pretreatment There was a significant increase in the incidences of simple and papillary or nodular hyperplasia in the urinary bladder (simple hyperplasia: 27/29 vs. 19/28; papillary or nodular hyperplasia: 24/29 vs. 11/28).	Nakanishi et al. (1982)
6.6.2 2-Acetylaminofluorene (AAF)							
Horton Sprague-Dawley rats (age not specified)	62F	62F	sodium saccharin, methods of production and purity not specified	5% diet	40 wk	Positive with co-administration of AAF Hyperplasia of the urinary bladder mucosal lining occurred in all animals but was more severe in AAF/sodium saccharin-exposed animals, with one of these animals displaying squamous metaplasia and precancerous changes in the mucosal epithelium. No animals had malignant lesions of the urinary bladder. IARC noted the small number of animals used and the fact that food consumption was not measured, preventing the evaluation of AAF and sodium saccharin exposure.	Ershoff and Baja (1974; cited by IARC, 1980)
F344 rats (age not specified)	31M	30M (BBN alone)	sodium saccharin [7 ppm *o*-toluenesulfonamide]; methods of production and purity not specified	0.02% AAF in diet for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Positive with AAF pretreatment There was a significant increase in the incidences of simple and papillary nodular hyperplasia of the urinary bladder (simple hyperplasia: 6/29 vs. 0/28; papillary or nodular hyperplasia: 4/29 vs. 0/28).	Nakanishi et al. (1982)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6.6.3 *N*-Methyl-*N*-nitrosurea (MNU)							
Wistar rats (age not specified)	63F (MNU + sodium saccharin containing 40 mg/kg *o*-toluene-sulfonamide) 63F (MNU + sodium saccharin free of *o*-toluene-sulfonamide)	63F (MNU alone)	MNU sodium saccharin prepared by the Remsen-Fahlberg method, containing 40 mg/kg *o*-toluene-sulfonamide sodium saccharin prepared by the Maumee process (no *o*-toluenesulfonamide)	0.15 mL instilled into bladder 2 g/kg/day in drinking water 2 g/kg/day in drinking water	single dose 2 yr (started 2 wk after MNU) 2 yr (started 2 wk after MNU)	Positive with MNU pretreatment There was an increase in the number of proliferative bladder lesions in rats treated with MNU and sodium saccharin (incidence not given).	Hooson et al. (1980)

Abbreviations: F = females; LI = labeling index; M = males

7.0 MECHANISMS

Summary: Bladder tumors found predominantly in male rats exposed to high dietary concentrations of sodium saccharin (equal to or greater than 1% of the diet) prior to birth, at birth, or starting at up to 35 days of age are thought to occur and proceed in association with elevated urinary sodium ion concentration and pH above 6.5. Implications that the sodium ion, itself, may be at least partially responsible for the carcinogenic effects observed in the male rat bladder stem from studies involving many other sodium salts (e.g., of succinic acid and ascorbic acid) eliciting similar effects in the male rat. In addition, when rat bladder epithelial cells were incubated with sodium saccharin, calcium saccharin, potassium saccharin, sodium ascorbate, sodium chloride, sodium citrate, potassium chloride, or calcium chloride *in vitro* for 24 hours, all of the sodium salts proved to be cytotoxic, while the other salts did not display similar effects. Studies using diets varying in pH have shown that sodium saccharin does not significantly promote proliferation in the male rat urinary bladder when fed in the acidic AIN-76A diet, but sodium saccharin did increase urothelial proliferation when fed in the Prolab 3200 (alkaline) diet.

A number of studies have shown that pH above 6.5 and increased urinary sodium ion concentration in the male rat urinary bladder enhance the formation of urinary silicate crystals. These crystals have been shown to form by the binding of urinary proteins to saccharin, and may act as microabrasives in the rat urinary bladder, causing regenerative hyperplasia (increase in cell number) and increased cell proliferation, which, when sustained over a lifetime, provide the basis for urinary bladder tumorigenesis. The anatomy of the rat bladder is thought to play a role in rendering the rat susceptible to bladder tumorigenesis. It is known that the horizontal position of the rat during urination leaves the rat prone to the retention of calculi in the bladder, and the formation and retention of precipitate in the rat bladder has been linked to the induction of tumors predominantly in the male rat.

Other factors associated with induction of urinary bladder tumors in the rat include high urine volume, low urine osmolality, and intrinsically high urinary protein, especially in male rats. It is noteworthy that saccharin binds to urinary proteins, including $\alpha 2\mu$-globulin which is common in male rats, and that the most extensive mechanistic studies have been conducted only in male rats. Whether the female rat positive urinary bladder response seen in initiation/promotion studies is associated with increased urinary protein and urinary crystal formation has not been adequately studied. Furthermore, extensive mechanistic studies in mice exposed to high doses of sodium saccharin, with or without previous exposure to a urinary bladder initiator, have not been done to definitively rule out the possibility that mice could also develop urinary bladder neoplasia under specific experimental conditions.

The constellation of physiological characteristics of urine in rats fed high doses of sodium saccharin, particularly commencing at times when intrinsic bladder urothelial proliferation is high, would not be expected in humans exposed to normal usage levels of sodium saccharin.

7.1 Mechanism of Urinary Bladder Tumorigenesis Found Predominantly in Male Rats

Long-term studies of sodium saccharin have shown that bladder tumors are the most common malignancies and that they occur predominantly in the male rat. Tumors found in the bladder are detected only when sodium saccharin is fed at high dietary levels (equal to or greater than 1% in rats) beginning at birth or when fetal rats are exposed *in utero* by feeding the dams 5% sodium saccharin diet (Schoenig et al., 1985; for review, see Velazquez et al., 1996). Schoenig et al. (1985) also found that *in utero* exposure was not necessary and that the incidence of bladder tumors in rats given 5% sodium saccharin from birth was essentially identical to that in rats fed 5% sodium saccharin prior to conception and throughout life (for review, see Renwick, 1993; Williams and Whysner, 1996).

Cohen et al. (1991b) offered the following hypothesis to describe the events leading to urinary bladder tumorigenesis in male rats: When sodium saccharin is fed to male rats at high dietary levels (about 2.5%), the concentration of urinary sodium is increased and the pH level is elevated (above 6.5). Under these conditions, binding of saccharin and male-rat-specific α2μ-globulin results in the formation of silicon-containing crystallized precipitate in the bladder (for review, see Ellwein and Cohen, 1990; Burin et al., 1995a; Cohen et al., 1995d; Velazquez et al., 1996). After binding, the precipitate enters the bladder urothelial cells and is cytotoxic. Acting as microabrasives, the silicate and precipitate particles irritate the mucosa and cause focal necrosis. The loss of urothelial cells results in regenerative hyperplasia and increased cell proliferation, which, when sustained over the rats' lifetime, provides the basis for urinary bladder tumorigenesis. Cohen et al. (1991a) further hypothesized that diet-, dose-, species-, and sex-specific effects of saccharin may be related to the formation of the particles (for review, see Burin et al., 1995a; Velazquez, 1996).

7.1.1 The Role of pH in the Promotion of Bladder Carcinogenesis in Male Rats

Studies indicate that a urinary pH higher than 6.5 promotes the tumorigenicity of sodium saccharin in male rats (for reviews, see Murai et al., 1997; Cohen et al., 1995d). For instance, Okamura et al. (1991) compared the effects of sodium saccharin on 5-wk-old male F344 rats initiated with 0.2% FANFT for 4 weeks followed by administration for 100 weeks of either 0 or 5% sodium saccharin in either Prolab 3200 or AIN-76A diet. In rats, administration of AIN-76A diet results in a strongly acidic urine, with a pH lower than 6.0 (for review, see Cohen, 1995c; Velazquez et al., 1996) while Prolab 3200 produces a neutral or slightly alkaline urinary pH (Fisher et al., 1989). [Humans tend to have acidic urine, with a pH between 5.0 and 6.0, although diet can alter this (Cohen, 1995c)]. The data from the study by Okamura et al. (1991) demonstrated that sodium saccharin did not significantly promote urinary bladder tumors in the male rat if fed an AIN-76A diet. However, there was a significant increase the incidence of bladder tumors if male rats were fed the Prolab 3200 diet.

A study by Garland et al. (1989b) also evaluated the responses of 5-wk-old male F344 rats to sodium saccharin administered in different diets. However, while Okamura et al. (1991) used tumor formation as an endpoint, Garland et al. (1989b) looked only at cellular proliferation in the urinary bladder, presumably because of the short duration of the study (10 weeks). Rats were either administered 0 or 7.5% sodium saccharin in Prolab 3200, AIN-76A, or NIH-07 diet and killed after 4 weeks, or they were administered 0, 5, or 7.5% sodium saccharin in these same

diets and killed after 10 weeks. In rats killed after 4 weeks of treatment, there was a significantly higher incidence of hyperplasia with administration of sodium saccharin and this incidence was higher in rats fed the Prolab diet than in rats fed the NIH diet. There was little response when sodium saccharin was administered in the AIN-76A diet. In the group of rats killed after treatment for 10 weeks, there was a similar trend (these rats also demonstrated a dose-dependent increase in hyperplasia). Since the urinary pH of rats fed sodium saccharin in the NIH-07 diet is known to be slightly lower than the urinary pH in rats fed Prolab 3200, and the urinary pH of rats fed AIN-76A is known to be even lower than that of rats fed NIH-07 (ibid.), these results are consistent with the hypothesis that urinary pH participates in the mediation of the proliferative response in urinary bladders of male rats exposed to sodium saccharin.

The findings of Okamura et al. (1991) and Garland et al. (1989b) imply that alkaline urinary pH alone was responsible for mediating urothelial proliferation, but other factors might also explain this phenomenon. For instance, while different diets have been shown to produce different urinary pH levels, they also can produce different levels of ions such as calcium, potassium and sodium, and silicates (Cohen, 1995c). Other studies, however, have supported a role for urinary pH in saccharin-induced carcinogenesis, showing that a pH above 6.5 greatly enhances the formation of the bladder epithelium-irritating urinary silicate crystals in male rats fed sodium saccharin (for review, see Cohen et al., 1991a). For a review of the role of pH in oncogenesis, see Harguindey et al. (1995).

7.1.2 The Role of Sodium Concentration in the Promotion of Bladder Carcinogenesis in Male Rats

There is evidence indicating that induction of bladder carcinogenesis in male rats exposed to saccharin is increased under conditions of high urinary sodium ion concentration. For instance, Hasegawa and Cohen (1986) fed weanling male F344 rats the sodium, potassium, or calcium salt of saccharin, or acid saccharin as 5% of the diet for ten weeks. They found that sodium saccharin induced a significantly higher level of urinary bladder epithelial proliferation than potassium saccharin. Calcium saccharin and acid saccharin, on the other hand, did not significantly change the bladder epithelium. Anderson et al. (1988) found similar results in weanling male CD rats. Like Hasegawa and Cohen (1986), they fed sodium saccharin, potassium saccharin, calcium saccharin, or acid saccharin to rats for 10 weeks and noticed that only sodium saccharin and potassium saccharin produced hyperplasia in the bladder. In a later study by Cohen et al. (1991b), after a 6-wk initiation period with 0.2% FANFT, sodium saccharin, administered as 3% or 5% of the diet for 72 weeks, was shown to be tumorigenic in male F344 rat bladders while calcium saccharin was only slightly so and acid saccharin was not at all.

In a review written by Cohen et al. (1997), it was noted that in rats, oral administration of sodium saccharin causes an increase in cell proliferation in the urothelium that is more pronounced than that induced by potassium saccharin, whereas calcium saccharin produces only slight changes and acid saccharin has no effect on the urinary bladder. It was also noted that these differences in potency occur even though urinary saccharin concentrations do not vary greatly among rats administered the different forms of saccharin. Refer to **Table 7-1** for a summary of the effects of various forms of saccharin on the rat urinary bladder. Refer to **Table 7-2** for results of urine analyses in rats given various forms of saccharin.

While sodium saccharin has been shown to induce carcinogenesis in the male rat bladder, so have many other sodium salts including those of vitamin C (Fukushima et al., 1986), glutamate and bicarbonate (for review see Cohen, 1995b), and succinic acid (Otoshi et al., 1993) (most of these studies did not evaluate the responses of female rats). This implies that the sodium ion, itself, may be at least partially responsible for these effects. Studies supporting this idea include those by Shioya et al. (1994) and Shibata et al. (1989), both of which only evaluated the responses of male rats. A list of sodium salts that produce changes in the rat bladder is provided in **Table 7-3**.

The results of an *in vitro* study performed by Garland et al. (1989a) suggest that the carcinogenic effect on the bladder of a high urinary sodium ion concentration could be mediated by the cytotoxicity of these ions. Transformed rat bladder epithelial cells (sex of donor animals not specified) were incubated in sodium saccharin, calcium saccharin, potassium saccharin, sodium ascorbate, sodium chloride, sodium citrate, potassium chloride, or calcium chloride for 24 hours and then attachment and viability of the cells were assessed. All of the sodium salts (and potassium saccharin) decreased cell attachment and viability, while potassium chloride and calcium chloride did neither. Calcium saccharin decreased only cell viability.

Another possible mechanism for sodium-induced carcinogenesis is direct induction of cellular proliferation and/or DNA synthesis by sodium ions (for review, see Cohen, 1995c). Several *in vitro* studies support this hypothesis. For example, Burns and Rozengurt (1984) used confluent quiescent Swiss mouse 3T3 cells to demonstrate that initiation of DNA synthesis in these cells by various stimulants was inhibited by limiting extracellular sodium ion concentration. Normally, 3T3 cells will initiate DNA synthesis when growth factors are included in their incubation media. However, when Burns and Rozengurt (1984) included one growth factor (i.e., epidermal growth factor, vasopressin, or insulin) in the media (serum-free), and removed extracellular sodium ions, there was no initiation of DNA synthesis.

Another study by Cameron et al. (1980) evaluated intracellular sodium ion concentrations in slowly and rapidly dividing cells, and in tumor cells. They found that sodium ion concentrations were highest in tumor cells and lowest in slowly dividing cells. They concluded that high sodium ion concentrations were, associated with mitogenesis while very high levels were associated with oncogenesis. However, the studies do not necessarily provide any support for the hypothesis that extraneous high sodium ion concentrations were responsible for induction of cellular proliferation or oncogenesis.

The most likely mechanism for a carcinogenic response to sodium saccharin mediated by sodium ions is the interation of sodium ions with proteins in the urine (Cohen, 1995c). It has been shown that urinary proteins in rats bind to saccharin to produce a crystallized precipitate (Cohen, 1995b; Cohen et al., 1995a), which may act as an abrasive in the rat bladder, causing regenerative hyperplasia (Cohen et al., 1990; Hicks, 1984). The formation of this precipitate is greatly enhanced by high sodium ion concentrations (Cohen et al., 1991a), thus raising the possibility that high sodium ion concentration is a necessary condition for precipitate formation.

Renwick (1993) stated that the urinary concentration of the anion of sodium saccharin does not play a role in the overall mechanism for tumorigenesis in the rat bladder. In addition, Renwick (1993) suggests that dietary sodium saccharin provides a vehicle for the delivery of "massive" but non-toxic amounts of sodium ions to the urinary bladder. However, the sodium

ion concentrations in the feed containing carcinogenic doses of sodium saccharin are not much higher than in the rat feed alone. For example, Purina Rodent Chow consists of 0.3% sodium ions or 3000 ppm. When comparing this concentration to the highest sodium saccharin concentration known to promote tumorigenesis (7.5% or 75,000 ppm), we calculated that the sodium ion concentration in the feed at this dose was approximately 3-fold that found in a typical rat chow (75,000 ppm x 12.5% sodium ions in sodium saccharin = 9400 ppm). Although a 7.5% sodium saccharin diet increased the concentration of sodium ions approximately 3-fold, this concentration scarcely represents a large increase from the usual daily dietary intake of sodium ions.

7.1.3 The Combined Effect of pH Level and Sodium Concentration

While both pH and sodium ions have been shown to affect cell proliferation in the bladder, most likely these two parameters do not act in isolation but are part of a set of parameters that regulate tumorigenesis. This hypothesis is supported by a study conducted by Fukushima et al. (1988) in which male F344 rats were initiated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and then fed a diet containing either 3% sodium bicarbonate, 1% sodium chloride, or a control diet. Sodium bicarbonate was found to increase urinary pH and sodium ion concentration and promote urinary bladder carcinogenesis. Administration of sodium chloride produced an increase in urinary sodium ions but not pH, and did not promote urinary bladder carcinogenesis.

Ito and Fukushima (1989) also found that both elevated pH and elevated sodium ion concentration were necessary conditions for induction of bladder tumorigenesis. They initiated male rats with 0.05% BBN and then administered either ascorbic acid, sodium ascorbate, sodium bicarbonate, or ammonium chloride alone or in several different combinations. Promotion of urinary bladder carcinogenesis occurred only under conditions of both elevated urinary pH and elevated urinary sodium ion concentration, induced by the administration of sodium bicarbonate and sodium ascorbate.

7.1.4 The Association Between Increased Urinary Output and Sodium Saccharin-Induced Bladder Tumors

Schoenig et al. (1985) found that rats that ingested 7.5% sodium saccharin in a two-generation bioassay and developed bladder tumors had a higher urine volume throughout their lives than did those that did not develop bladder tumors. Schoenig et al. (1985) also found that the difference in urine volume between the non-tumor bearing group and the untreated controls was almost as great as the difference between the sodium saccharin-treated tumor bearing and non-tumor bearing rats.

Anderson et al. (1987b) studied the effect of inherent urine output (high urine volume or low urine volume) on the response of male rats fed 7.5% dietary sodium saccharin for 10 weeks. Rats exposed to 7.5% dietary sodium saccharin for 10 weeks showed an increased incidence of bladder epithelial hyperplasia (12/20 rats exposed to sodium saccharin vs. 2/20 controls). The incidence of hyperplasia was similar (6/10) in the sodium saccharin high and low urine output groups. One of the two control rats that had hyperplastic lesions in the bladder showed evidence

of inflammation and had a higher than average urine output, while the other had the highest urine output in the control group (73 g/kg).

Anderson et al. (1987a) found that high urine output rats in the control group consumed more feed than those in the low urine output control group. Therefore, the authors compared the mean daily sodium saccharin consumption between the exposed high and low urine output groups (g/kg bw) for the 10-week period. The authors found that the high urine output group consumed 8.0 ± 0.2 g/kg bw and the low urine output group consumed 7.8 ± 0.2 g/kg bw feed containing 7.5% sodium saccharin on an average daily basis. However, urinary concentrations of saccharin were similar in the high and low urine mass groups (41 ± 3 and 46 ± 2 mg/mL, respectively). Thus, Anderson et al. (1987a) concluded that it is unlikely that a difference in urinary saccharin concentration or total saccharin exposure can account for the role of high urinary volume in saccharin-associated bladder tumorigenicity.

7.2 Dose Response in Cell Proliferation and Tumorigenesis

Numerous studies have been conducted that suggest high doses of sodium saccharin produce urinary bladder tumors in male rats. For example, Cohen et al. (1991b) and Fukushima et al. (1986) have demonstrated that the effects of high dietary concentrations of sodium saccharin on male rat bladder epithelium are associated with increased urinary bladder tumor promotion. Cohen et al. (1989 abstr.) found that feeding male rats high doses of sodium saccharin (7.5%) beginning 5 weeks after birth increased cell proliferation in the bladder urothelium. Cohen and Ellwein (1991) suggested that approximately one-third of the total mitoses of the urothelium occurs within the first 3 weeks of a rat's life. Therefore, when sodium saccharin dosing begins at birth, rather than after weaning, rats are somehow more susceptible to sodium saccharin-induced tumors in later life due to the increased cell proliferation occurring at this time. The increases of cell proliferation observed after short periods of high sodium saccharin administration are dose-responsive. Details of carcinogenesis experiments mentioned herein are in **Table 4-1**.

Schoenig et al. (1985) conducted a 2-generation rat bioassay on sodium saccharin. This study involved 2500 second-generation male Charles River CD rats (F_1, between 21 and 38 days of age; 6 treatment groups, 125 to 700 rats per group) receiving 1, 3, 4, 5, 6.25, and 7.5% sodium saccharin in their diet for up to 30 months. The parents (F_0) of the F_1 generation had been maintained on diets containing between 1 and 7.5% sodium saccharin. Except during mating, gestation, and lactation, all animals were housed individually in a single environmentally controlled room. The data resulting from this experiment, designed to determine the dose-response for urinary bladder tumors, indicated that a 1% dietary level of sodium saccharin represented a no-effect level. Higher dietary concentrations showed a very steep dose-response, indicating that tumor incidence increased rapidly with an increase in the dose. For example, significant increases in the incidence of primary neoplasia (benign and malignant tumors) in the urinary bladder of F_1 male rats sacrificed during month 15 of this study were not found in the 1.0 or 3.0% sodium saccharin group. However, pairwise comparisons between the control group (0.0% total primary neoplasia) and all groups treated with 4, 5, 6.25, and 7.5% sodium saccharin showed significant increases in the incidence of benign (2.1, 3.3, 10.0, and 15.3%, respectively) and malignant tumors (4.2, 9.2, 6.7, and 16.1%, respectively) alone as well as of total primary

bladder neoplasia (6.3% to 31.4%). Total primary bladder neoplasia was also significantly higher in the 3.0% sodium saccharin group (1.7%). Therefore, the 1.0% sodium saccharin dietary level was considered to be a no-effect level for bladder neoplasia. However, 5 bladder tumors were found in the 1% sodium saccharin group and none were found in the concurrent controls. This finding prompted the authors to review the historical control data for the incidence of primary urinary bladder tumors in male Charles River CD rats at IRDC (Squire, 1985). The review included 10 studies that examined the urinary bladders from 982 male controls either *in utero* or over a lifetime. Of these animals, 863 survived for 67 weeks, which corresponds to the appearance of the first urinary bladder tumor observed in the bioassay conducted by Schoenig et al. (1985). No primary urinary bladder tumors appeared prior to week 67 in the controls of the ten studies reviewed. The percentage incidence of tumors was calculated from historical controls by using the number of rats that survived until the first bladder tumor was observed as the denominator. These data showed that total primary bladder neoplasia ranged from 0.0 to 3.3% with a mean of 0.8%. The corresponding incidence of total primary bladder neoplasia at the 1% dietary sodium saccharin observed by Schoenig et al. (1985) was also 0.8%. These findings suggest that the NOEL (1% sodium saccharin dietary level) proposed by Schoenig et al. (1985) is not significantly different from the results obtained from the controls (0.8%) studied by Squire (1985), and that the background tumor incidence for this strain of rat at IRDC was identical to that observed in the 1% sodium saccharin group (0.8%) studied by Schoenig et al. (1985).

Murusaki et al. (1981), who studied the light microscopic and electron microscopic changes in the bladder of rats fed sodium saccharin (dietary concentrations between 0.1 and 5%), also reported a steep dose-response curve over a narrow range of dose levels above 1%. Furthermore, Nakanishi et al. (1980a) and West et al. (1986), using light microscopy, autoradiography, and scanning electron microscopy, detected cellular responses in male rat bladders only with sodium saccharin dietary concentrations of 2.5% to 5% beginning at 6 to 8 weeks of age. Chappel (1992) reviewed and assessed the biological risk of sodium saccharin. The author stated that the steep dose-response curves representing both physiological changes in the urine and morphological changes in the urothelium provide strong evidence of a common threshold at a sodium saccharin dietary concentration between 1 and 3%. To Chappel (1992), these results provided strong evidence that these phenomena are interrelated.

Ellwein and Cohen (1988), using model-based simulations, demonstrated that the proliferative effects (hyperplasia; increase in LI) following high doses of sodium saccharin are sufficient to explain tumorigenic effects in the rat urinary bladder without having to postulate a genotoxic influence. Their database was generated from a large series of experiments dealing with the increase in LI and hyperplasia after the administration of high doses of sodium saccharin. The authors postulated a tumorigenic effect secondary to sodium saccharin administration only if it is administered during the neonatal period at a dose which will generate a cell proliferative response in the urothelium; and after weaning when ulcerations of the bladder occur. The authors suggested that a dietary level of at least 1% sodium saccharin is necessary for a cellular response to occur in the rat bladder, even though most experiments aimed at cellular responses detected by light microscopy, autoradiography, or scanning electron microscopy (West et al., 1986; Murusaki et al., 1981; Nakanishi et al., 1980a) have found these effects only at doses of 2.5% or higher.

Like Chappel (1992), Ellwein and Cohen (1990) suggested that saccharin exhibits a biological threshold.

7.3 Relevance of Animal Cancers To Humans

Numerous studies have investigated the carcinogenicity of sodium saccharin in rats (Cohen et al., 1995b; Cohen et al., 1990; Anderson, 1988; for review, see Oser, 1985; Williams and Whysner, 1996), mice (for review, see Oser, 1985), non-human primates (Thorgeirsson et al., 1994) and humans (Risch et al., 1988; for reviews, see Elcock and Morgan, 1993; Chappel, 1992; Ellwein and Cohen, 1990; Morgan and Wong, 1985). These studies have revealed that it is mainly the male rat which is susceptible to the formation of bladder tumors following chronic exposure to high doses of sodium saccharin (Cohen, 1995b; Chappel, 1992), i.e., greater than or equal to 1% of the diet (Ellwein and Cohen, 1990). A summary of positive mammalian carcinogenicity studies is presented in **Table 7-4**. An interspecies comparison of the effects of sodium saccharin on the urinary bladder is presented in **Table 7-5**, and a comparison of the effects of sodium saccharin in various rat strains is presented in **Table 7-6**.

Results from animal studies suggest that there is an intrinsic difference between male rats and other animals in how they react to sodium saccharin exposures and, in particular, they imply that there may be a peculiarity of the male rat bladder which makes the male rat uniquely susceptible to cancer of this organ following sodium saccharin exposures. Most likely, this peculiarity is not of a genetic origin but is, rather, physiologically based (Weisburger, 1990), since sodium saccharin has been shown to be non-genotoxic *in vivo* (Ellwein and Cohen, 1990; Ashby, 1985; Lutz and Schlatter, 1977).

If the male rat bladder is indeed a unique organ with respect to its response to sodium saccharin, it would have to be concluded that male rats do not accurately represent humans when considering such a response and that it would therefore not be appropriate to extrapolate data from male rat exposure studies to humans. This section will investigate the validity of these statements by comparing the anatomy and physiology of the male rat bladder with the human bladder.

7.3.1 Comparative Bladder Anatomy and Urine Chemistry

The anatomy of the rat bladder is significantly different than that of the human bladder. For instance, the rat bladder is an abdominal organ, while the human bladder progresses from an abdominal organ in infancy and childhood to a pelvic organ in adulthood when the pelvis is fully developed and upright posture of the body is achieved (DeSesso, 1995).

The upright/vertical posture of mature humans versus the horizontal posture of rats is highly relevant to the nature of bladder response to sodium saccharin when the process of urination is considered. Specifically, it is known that the vertical position of humans allows for a more efficient elimination of calculi from the bladder while the horizontal position of the rat during urination leaves the rat more prone to retention of such material (Burin et al., 1995b; Cohen, 1995b).

Although other animals (e.g., mice) that maintain a horizontal position may also be susceptible to calculus retention, this phenomenon is uniquely relevant to rats when exposure to sodium saccharin is considered. This is due to the fact that sodium saccharin has been shown to

induce precipitate formation solely in male rat urine (see **Table 7-5**) (Cohen, 1995b; Cohen et al., 1995a; Cohen et al., 1991a), and the formation and retention of this precipitate has been linked to the formation of tumors of the male rat bladder (Cohen 1995b). Tumor formation may be the result of chronic irritation, and the damage it causes to bladder urothelium (Burin et al., 1995b; Clayson et al., 1995; Ellwein and Cohen, 1988). The precipitate is composed of mainly calcium phosphate, but also contains silicate, protein, saccharin, sulfur-containing substances, potassium, and chloride (Cohen, 1995b), and is jagged in nature (Cohen et al., 1989 abstr.).

When the urothelium is damaged by abrasion, regenerative hyperplasia is likely to occur (Cohen et al., 1990; Hicks, 1983). This results in an increase in the number of urothelial cell divisions (Cohen and Lawson, 1995) which may lead to tumor formation (Cohen and Ellwein, 1991).

After sodium saccharin exposure, the formation of precipitate in the male rat urine is thought to be the result of an interaction in the urine between saccharin and the male rat-specific protein, α2μ-globulin (Murai et al., 1997; Garland et al., 1994; Swenberg et al., 1992). Alpha 2μ-globulin is a low-molecular-weight protein, weighing less than 40 kDa (Hard, 1995). It is synthesized in the liver and is quantitatively the major protein found in male rat urine (Roy and Neuhaus, 1966). It is not present in significant quantities in female rat urine and is not synthesized by humans (Hard, 1995).

It has been shown that rats lacking α2μ-globulin are not as subject to bladder cell proliferation following sodium saccharin exposure as are rats producing this protein. Uwagawa et al. (1994) used the male NBR rat, which does not synthesize α2μ-globulin, and the male F344 rat, which does, to demonstrate this. After chronic administration (starting at 6 weeks of age) of a diet containing 5% sodium saccharin, the F344 rat showed signs of cellular proliferation in the bladder urothelium, but the NBR rat did not.

A study by Garland et al. (1994) supports the findings of Uwagawa et al. (1994). Four- to 5-week-old intact F344, castrated F344, and NBR rats were administered 7.5% sodium saccharin in the diet for 10 weeks. Less cellular proliferation occurred in the bladders of the castrated rats, which had reduced levels of α2μ-globulin, than in the bladders of intact F344 rats. Even less proliferation was seen in the bladders of NBR rats, which had lower levels of α2μ-globulin than the castrated rats.

Since α2μ-globulin is normally specific to the male rat and since this protein is thought to be at least partially responsible for the carcinogenicity of sodium saccharin in the bladder, α2μ-globulin in the urinary bladder is probably the physiologic peculiarity that renders the male rat bladder susceptible to a carcinogenic response to sodium saccharin (for review, see Swenberg et al., 1992). However, it is important to note that while Uwagawa et al. (1994) and Garland et al. (1994) demonstrated an association between the presence of α2μ-globulin in the male rat bladder and the occurrence of cellular proliferation of the bladder, no studies were found which evaluated the role of α2μ-globulin in the formation of tumors in these animals.

It is also noteworthy that saccharin binds to other proteins besides α2μ-globulin and that most extensive mechanistic studies have been conducted only in male rats. Whether the female

rat positive bladder response seen in I/P studies is associated with an increase in protein has not been studied.

Another problem with the accuracy of this hypothesis arises from integrating studies investigating the critical age of sodium saccharin administration to male rats for induction of bladder tumors with those investigating the age-dependent expression of α2μ-globulin. It is thought that sodium saccharin produces urinary bladder tumors in male rats only if it is administered before the rats reach 35 days of age (Cohen et al., 1995b) unless exposure occurs after administration of an initiating agent (for review, see Cohen et al., 1995a). In several studies in which rats were exposed to sodium saccharin beginning after this time period, there was no increase in the incidence of bladder tumors in male rats (e.g., Homma et al., 1991; Murasaki and Cohen, 1981; Hooson et al., 1980; Schmähl, 1973; cited by IARC, 1980; for reviews, see Cohen and Ellwein, 1991a; National Academy of Sciences-National Research Council, 1974; cited by Arnold et al., 1980). It has also been shown that hepatic synthesis of α2μ-globulin in the male rat does not begin until 35 to 40 days of age (Roy et al., 1983) and is thus undetectable in male rats below this age (Neuhaus and Flory, 1978). Therefore, the time of susceptibility to induction by sodium saccharin of cellular proliferation in the bladders of male rats does not correlate with the presence of α2μ-globulin in these rats. While this does not necessarily preclude a role for α2μ-globulin in sodium saccharin carcinogenesis, it does raise some doubts.

While the possibility of a role for α2μ-globulin in sodium saccharin carcinogenesis is attractive because it can account for differences in species (e.g., rat and human) and sex (e.g., male and female rats) responses to sodium saccharin, other mechanisms of sodium saccharin carcinogenesis could exist that would also successfully explain these differences. For instance, proteins other than α2μ-globulin may be responsible for the unique vulnerability of male rats to sodium saccharin-induced bladder tumorigenesis. Since male rats have up to 10 times more total protein in their urine than female rats (Lehman-McKeeman and Caudill, 1991) and about 90 times more total urinary protein than humans (Olson et al., 1990), the idea that a protein other than α2μ-globulin can account for species and sex differences in sodium saccharin response is not implausible (for an interspecies comparison of urine chemistries see **Table 7-7**). Few studies have investigated this hypothesis, although the role of albumin was examined by Homma et al. (1991). This group compared the response of analbuminemic rats to sodium saccharin exposure to the response of Sprague-Dawley rats. Neither strain developed abnormal bladder growths and the study was inconclusive. Since albumin levels in humans are known to be higher than levels in male rats (Hard, 1995), future studies should probably focus on investigating low-molecular-weight proteins other than α2μ-globulin that are more abundant in male rats than in female rats or humans (Olson et al., 1990).

7.3.2 Dose-Response Extrapolation

Two major issues to consider when deciding if dose-extrapolation from rats to humans is appropriate are the nature of the carcinogenic mechanism (i.e., does it operate in both rats and humans?) and the presence or absence of a threshold in dose-response. In fact, the majority of data summarized in previous sections of this document indicate that the carcinogenic mechanism of sodium saccharin may be unique to male rats, and that there is a threshold dose.

There is significant data indicating that the mechanism of sodium saccharin-induced bladder carcinogenesis in male rats is related to the formation and retention of urinary precipitate formed under conditions of high urinary pH and high sodium concentration and that this precipitate does not form in other species.

Studies using diets varying in pH have shown that sodium saccharin does not significantly promote proliferation in the male rat urinary bladder when fed in the acidic AIN-76A diet, but sodium saccharin does increase urothelial proliferation when fed in the alkaline Prolab 3200 diet. It has also been shown that in rats, oral administration of sodium saccharin causes an increase in cell proliferation in the urothelium that is more pronounced than that induced by potassium saccharin, whereas calcium saccharin produces only slight changes, and acid saccharin has no effect on the urinary bladder, even though urinary saccharin concentrations do not vary greatly between the different groups of rats. In addition, while sodium saccharin has been shown to induce carcinogenesis in the male rat bladder, so have other sodium salts.

There is also evidence from a number of studies that a threshold dose exists in male rats for sodium saccharin-induced bladder carcinogenesis, suggesting that use of a linear dose-response model is not appropriate to estimate risk in humans.

7.4 Additional Mechanistic Information

7.4.1 Inhibition of Apoptosis (Programmed Cell Death)

Wright et al. (1994) reported that pretreatment with saccharin inhibited apoptosis (specifically the DNA fragmentation induced by UV light or tumor necrosis factor) in human histiocytic (U937) lymphoma cells.

7.4.2 Intercellular Communication

A review by IARC (1987a,b) reported that saccharin (form unspecified) inhibited intercellular communication in mammalian cells *in vitro* in two studies but not a third. These studies administered doses that were 1/2 those used in the tumor-positive rat studies. In a later review by Klaunig and Ruch (1990), the authors reported that saccharin inhibited intercellular communication in Chinese hamster lung V79 cells but not in primary mouse hepatocytes.

Table 7-1. Effect of Various Forms of Saccharin on the Rat Urinary Bladder

Treatment[a]	Simple Hyperplasia	Microvilli on SEM		Labeling Index (%)
		Uniform	Pleomorphic	
sodium saccharin	5/12[b]	2/6	2/6	0.55 ± 0.20 (5)[c]
potassium saccharin	2/12	2/6	0/6	0.18 ± 0.09 (6)[d]
calcium saccharin	2/12	1/6	0/6	0.12 ± 0.11 (6)
acid saccharin	0/12	0/6	0/6	0.07 ± 0.04 (6)
control	0/12	0/6	0/6	0.06 ± 0.04 (6)

[a] 5% in diet for 10 wk
[b] significantly different from acid saccharin and control group, $p < 0.02$
[c] significantly different from all other groups, $p < 0.01$
[d] significantly different from control group, $p < 0.05$

Source: Cohen (1994a)

Table 7-2. Urine Analysis in Rats Given Various Forms of Saccharin

Treatment[a]	Urine Volume (mL/day)	Saccharin (mmol/mL)	pH	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mEq/L)	Osmolality (mOsm/L)
sodium saccharin	10.4	0.17	7.2	291	151	24.8	1520
potassium saccharin	13.5	0.14	6.8	153	298	23.9	1463
calcium saccharin	6.3	0.14	5.7	158	236	41.2	2145
acid saccharin	8.8	0.19	5.5	139	164	51.6	2029
control	6.7	0	7.1	158	201	34.5	1678

[a] 5% in diet for 4 wk

Source: Cohen (1994b)

Table 7-3. Sodium Salts That Produce Urothelial Hyperplasia and Increase the Incidence of Bladder Tumors in Rats Fed High Doses (> 1%)

Sodium ascorbate
Sodium aspartate
Sodium bicarbonate
Sodium chloride
Sodium citrate
Sodium erythorbate
Sodium glutamate
Sodium phosphate
Sodium phytate
Sodium saccharin
Sodium succinate

Source: Cohen et al. (1997)

Table 7-4. Summary of Positive Mammalian Carcinogenicity Studies

Age, Strain, Species	Chemical Form	Effective Dose and Duration	Primary Tumor Location	Comments on Mechanism of Action	Reference
Mice					
'stock' mice (age not specified)	saccharin[a]	2 mg saccharin/8 mg cholesterol pellets implanted in urinary bladder lumina for 52 wk (1-generation study)	urinary bladder	The presence of the cholesterol pellet in the bladder had a promoting action; saccharin was an incomplete carcinogen.	Allen et al. (1957)
6-wk-old albino mice	saccharin[a]	1.5 g/kg in 1 mL distilled water, force fed for 1 yr (1-generation study)	thyroid	Mechanism unknown, but results are questionable because control incidence was not reported, statistical analysis was not performed, sample size was small, purity of saccharin was not reported, and results have not been replicated.	Prasad and Rai (1986)
18- to 19-wk-old BALB/c mice	sodium saccharin	5.0% diet for 117 wk (1-generation study)	Harderian gland	No dose-response demonstrated. Marginally significant for trend. Probably not applicable to humans, since they only have rudimentary Harderian gland.	Frederick et al. (1989)
Rats					
Charles River CD rats (age not specified)	sodium saccharin	7.5% in diet for 28 mo (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Taylor and Friedman (1974 abstr.)
Weanling SD rats (age not specified)	sodium saccharin	5% in diet for 100 wk (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Tisdel et al. (1974)
32-day-old SD rats	sodium saccharin	5% in diet for 90 days (adults) or ~700 days (pups) (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Arnold et al. (1980)
in utero Charles River CD rats	sodium saccharin	7.5% in diet for ≤ 2 yr (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Taylor et al. (1980)
6-wk-old ACI rats[b]	sodium saccharin	5% in diet for 12 mo (1-generation study)	urinary bladder	Mechanism specific to males fed high dose. *Trichosomoides crassicauda* infection enhanced sodium saccharin-induced cell proliferation in urinary bladder.	Fukushima et al. (1983)
6-wk-old F_0 and 28- to 38-day-old F_1 Charles River CD rats	sodium saccharin	3.0, 4.0, 5.0, 6.25, or 7.5% in diet for 30 mo (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Schoenig et al. (1985)

[a] No distinction was made between saccharin and its sodium salt
[b] At least half of these rats were infected with the bladder parasite *Trichosomoides crassicauda*

Table 7-5. Interspecies Comparison of the Effects of Sodium Saccharin on the Urinary Bladder

Species	Bladder Hyperplasia[a]		Bladder Carcinogenesis[a]		Bladder Promotion[b]	Urinary Precipitate[b]
	1 Generation	2 Generation	1 Generation	2 Generation		
Hamster	+ (0) - (1)	NE	+ (0) - (1)	NE	NE	NE
Mouse	+ (0) - (1)	NE	+ (2)[c] - (6)	NE	-	-
Rat	+ (17) - (7)	+ (1) - (0)	+ (1)[d] - (14)	+ (5) - (0)	+	+
Guinea Pig	+ (0) - (1)	NE	NE	NE	NE	NE
Monkey	+ (0) - (1)	NE	+ (0) - (4)	NE	NE	-

NE = not evaluated

[a] Number of positive (+) and negative (-) studies in parentheses; data summarized from Tables 4-1 and 6-1
[b] Adapted from Cohen (1994c)
[c] These two studies were equivocal.
[d] This study was equivocal.

Table 7-6. Interstrain Comparison of the Effects of Sodium Saccharin on the Rat Urinary Bladder[a]

Rat Strain	Bladder Hyperplasia		Bladder Carcinomas	
	1 Generation	2 Generation	1 Generation	2 Generation
ACI	+ (1)[b] - (0)	NE	+ (1)[b] - (0)	NE
Charles River CD	NE	+ (1) - (0)	+ (0) - (2)	+ (3) - (0)
F344	+ (10) - (4)	NE	+ (0) - (1)	NE
NBR[c]	+ (0) - (1)	NE	NE	NE
Osborne-Mendel	NE	NE	+ (0) - (1)	NE
Sprague-Dawley	+ (4) - (1)	NE	+ (0) - (4)	+ (2) - (0)
Wistar	+ (1) - (2)	NE	+ (0) - (6)	NE

NE = not evaluated

[a] Number of positive (+) and negative (-) studies in parentheses; data summarized from Tables 4-1 and 6-1
[b] This study was equivocal; at least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda.*
[c] NBR rats do not synthesize α2μ-globulin.

Table 7-7. Interspecies Comparison of Fresh Void Urine Chemistry

Species Treatment	pH	Protein (mg/mL)	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)	Creatinine (mg/dL)	Phosphorus (mg/dL)	Urea (mg/dL)	Chloride (mEq/L)	Magnesium (mg/dL)
Human										
Male	6.4 ± 0.23	0.02 ± 0.00	160 ± 18.8	63 ± 10.7	16.6 ± 3	119 ± 15.8	42 ± 7.7	780 ± 87	160 ± 17.2	9.2 ± 1.9
Female	5.8 ± 0.16	0.03 ± 0.01	140 ± 16.9	62 ± 10.1	11.9 ± 2	103 ± 17.3	42 ± 7.7	721 ± 114	162 ± 20.7	6.6 ± 1.3
Monkey-Cyano										
Control Male	7.2 ± 0.67	0.02 ± 0.02	15 ± 5.0	13 ± 11.0	4 ± 1.0	15 ± 6	1.5 ± 0.5	119 ± 98	15 ± 0	1.5 ± 0.5
NaSac Male	7.0 ± 0.56	0.09 ± 0.02	10 ± 0	47.5 ± 43.5	58 ± 52	71.5 ± 58.5	1 ± 0	468 ± 406	16 ± 1.0	14 ± 12
Control Female	6.7 ± 0.19	0.14 ± 0.13	46.5 ± 36.5	58.5 ± 29.5	55 ± 54	131 ± 102	1 ± --	792 ± 588	39 ± 24	11.5 ± 10.5
NaSac Female	6.8 ± 0.32	0.17 ± 0.07	16.5 ± 6.5	19 ± 7	13 ± 4	30 ± 7	2 ± 0	376 ± 114	14 ± 1	7.5 ± 3.5
Monkey-Rhesus										
Control Male	7.0 ± 0.11	0.13 ± 0.05	13.5 ± 3.5	21 ± 13	12 ± 2	25.5 ± 11.5	1 ± 0	219 ± 115	15 ± 0	5 ± 0
NaSac Male	7.0 ± 0.24	0.10 ± 0.03	15 ± 5	31 ± 25	10 ± 6	40.5 ± 24.5	1 ± 0	326 ± 249	25 ± 10	5.5 ± 3.5
Control Female	6.8 ± 0.75	0.07 ± 0.01	10 ± 0	11 ± 5	5.5 ± 0.5	18.0 ± 1.0	1 ± 0	138 ± 8.5	15 ± 0	2.5 ± 0.5
NaSac Female	6.5 ± 1.4	0.08 ± 0.08	10 ± 0	9.5 ± 7.5	3 ± 1	16 ± 6	1.5 ± 0.5	94 ± 61	15 ± 0	1.5 ± 0.5
Mouse										
Control Male	7.2 ± 0.07	1.10 ± 0.03	214 ± 22	216 ± 11.2	3 ± 0.38	20.2 ± 0.65	130 ± 6.5	2845 ± 131	218 ± 19	27.8 ± 3.5
Rat										
Control Male	6.8 ± 0.13	1.7 ± 0.08	199 ± 9.7	406 ± 19.4	6.8 ± 0.4	117 ± 4.0	223 ± 6.4	4069 ± 163.0	242 ± 11.6	29 ± 2.1
NaSac Male	6.5 ± 0.05	0.30 ± 0.05	251 ± 17.3	130 ± 7.5	12.5 ± 0.6	37 ± 2.6	136 ± 9.4	1328 ± 88.0	80.9 ± 6.0	43.6 ± 3.9
Control Female	7.2 ± 0.09	0.20 ± 0.06	222 ± 20.3	437 ± 38.7	14.8 ± 0.9	113 ± 7.0	250 ± 14.8	4281 ± 270.0	264 ± 22.5	36 ± 4.6
NaSac Female	6.6 ± 0.07	0.10 ± 0.02	274 ± 15.1	141 ± 3.7	14.9 ± 1.3	40 ± 1.7	142 ± 7.2	1591 ± 62.0	88 ± 3.4	56.3 ± 3.2

NaSac = sodium saccharin

Source: Cohen (1994d)

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APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 22 (Some Non-Nutritive Sweetening Agents) Saccharin, pp. 111-185, 1980

APPENDIX B

Excerpts from the IARC Monograph on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
Supplement 4 (Chemicals, Industrial Processes and Industries
Associated with Cancer in Humans, IARC Monographs Volumes 1 to 29)
Saccharin
pp. 224-226, 1982

APPENDIX C

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Supplement 7 (Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42) Saccharin pp. 334-339, 1987

APPENDIX D

Description of Online Searches for Saccharin and Saccharin Salts

DESCRIPTION OF ONLINE SEARCHES FOR SACCHARIN AND SACCHARIN SALTS

Initial online searches for saccharin [CASRN 128-44-9] and its sodium [81-07-2, anhydrous; 6155-57-3, dihydrate] and ammonium [6381-61-9] salts were performed in February and March 1996 in databases on the systems of STN International, DIALOG, NLM's TOXNET, and the Chemical Information System. Toxicology information was sought in the databases CCRIS (Chemical Carcinogenesis Research Information System), CHEMHAZIS (from the NTP Chemical Repository), EMIC, EMICBACK, GENETOX, RTECS (one record for each), and TOXLINE (name and CASRNs combined with terms for metabolism and the MESH heading for all neoplasms). Since that time, we have monitored 1200 life sciences journals for saccharin information using Current Contents on Diskette® (and cumulative issues on CD-ROM). We monitored not only for saccharin but also for information on rat bladder carcinogenesis induced by other chemicals and for articles by S. Cohen. We had requested and received many reprints on these topics by the time work resumed on this compound in 1997.

Market information, including production, shipments, sales and consumption, labor use, and workers by type was sought in IAC PROMT and the FOODLINE files Food Science and Technology and International Food Market Data in March 1996.

Regulatory information was sought in March 1996 from CHEMTOX and the FOODLINE file CURRENT FOOD LEGISLATION and more recently from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to the title 21 (FDA) and title 40 (EPA) regulations.

APPENDIX E

Listing of GAP Test Codes in Phylogenetic Order For Saccharin and Sodium Saccharin

LISTING OF GAP TEST CODES IN PHYLOGENETIC ORDER FOR SACCHARIN AND SODIUM SACCHARIN

Prokaryotic Systems:

PRB = Prophage, induction, SOS repair, DNA strand breaks or cross-links
SA5 = Salmonella typhimurium TA1535, reverse mutation
SA7 = Salmonella typhimurium TA1537, reverse mutation
SA8 = Salmonella typhimurium TA1538, reverse mutation
SA9 = Salmonella typhimurium TA98, reverse mutation
SA0 = Salmonella typhimurium TA100, reverse mutation

Lower Eukaryotic Systems:

SCG = Saccharomyces cerevisiae, gene conversion
SCH = S. cerevisiae, homozygosis by recombination or gene conversion
SCR = Saccharomyces cerevisiae, reverse mutation
SCN = Saccharomyces cerevisiae, aneuploidy
DMX = Drosophila melanogaster, sex-linked recessive lethal mutation
DMH = Drosophila melanogaster, heritable translocation test

Mammalian Systems in vitro:

DIA = DNA strand breaks, cross-links or rel. damage, animal cells in vitro
G5T = Gene mutation, mouse L5178Y cells in vitro, TK locus
SIC = Sister chromatid exchange, Chinese hamster cells in vitro
CIC = Chromosomal aberrations, Chinese hamster cells in vitro
TBM = Cell transformation, BALB/C3T3 mouse cells
TCM = Cell transformation, C3H10T1/2 mouse cells
TRR = Cell transformation, RLV/Fischer rat embryo cells
SHL = Sister chromatid exchange, human lymphocytes in vitro
CHL = Chromosomal aberrations, human lymphocytes in vitro

Mammalian Systems in vivo:

BFA = Body fluids from animals, microbial mutagenicity
DVA = DNA strand breaks, cross-links or rel. damage, animals in vivo
MST = Mouse spot test
SVA = Sister chromatid exchange, animal cells in vivo
MVM = Micronucleus test, mice in vivo
CBA = Chromosomal aberrations, animal bone-marrow cells in vivo
CGC = Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG = Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
DLM = Dominant lethal test, mice

*** Alternative test codes (not shown in profiles)**

BVD = Binding (covalent) to DNA, animal cells in vivo
ICR = Inhibition of intercellular communication, rodent in vitro
ICR = Inhibition of intercellular communication, rodent in vitro
ICR = Inhibition of intercellular communication, rodent in vitro
SPM = Sperm morphology, mouse

APPENDIX F

Listing from the Eighth Report on Carcinogens

SACCHARIN
CAS No. 128-44-9

First Listed in the *Second Annual Report on Carcinogens*

CARCINOGENICITY

There is sufficient evidence for the carcinogenicity of saccharin in experimental animals (IARC V.22, 1980; IARC S.4, 1982; IARC S.7, 1987). Saccharin is produced commercially as calcium and sodium salts (6485-34-3 and 128-44-9, respectively) as well as the free acid, and the name saccharin has been applied to all these chemicals. When saccharin was administered in the diet or drinking water, increased incidences of lymphomas/leukemias and transitional cell carcinomas of the urinary bladder were seen in rats. In multigeneration studies using rats, administration of saccharin in the diet induced transitional cell carcinomas and papillomas of the urinary bladder in the first generation male offspring. In one study when administered in the diet, saccharin induced papillary adenocarcinomas of the thyroid in mice. Several studies in which saccharin was administered orally to mice, rats, hamsters, and monkeys were considered inadequate for evaluation by IARC Working Groups. Surgical insertion of pellets containing saccharin resulted in urinary bladder cancer in mice and urinary bladder carcinomas in female mice. Other studies involving topical administration of saccharin to mice and intraperitoneal injection of female mice were considered to be inadequate for complete evaluation by IARC Working Groups. Transplacental exposure of rats to sodium saccharin and to saccharin did not produce any treatment-related neoplasms. Pretreatment with a single instillation in the urinary bladder of a low dose of *N*-methyl-*N*-nitrosourea or feeding of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and subsequent oral administration of sodium saccharin for long periods increased the incidence of urinary bladder neoplasms in rats over that induced by the nitrosourea or the amide alone. Simultaneous administration of *N*-nitroso-*N*-(4-hydroxybutyl)butylamine and sodium saccharin significantly enhanced the induction of urinary bladder papillomas over that seen after treatment with the nitrosamine alone.

An IARC Working Group reported that there is no adequate evidence for the carcinogenicity of saccharin in humans (IARC S.7, 1987). Since the positive report of Howe et al. (1980), the results of seven case-control studies and one population study of urinary bladder cancer have been inconsistent. The largest was a population-based study in 10 areas of the United States. Significant trends of increasing risk with increasing average daily consumption were found in female nonsmokers and male heavy smokers. Subsequent, independent reanalysis of the same data by a different statistical technique (multiple logistic regression) confirmed the original findings overall but cast doubt on the significance of the findings in the two subgroups because of inconsistent dose-response trends, especially among the male heavy smokers. Three other case-control studies have also shown increased risks among subgroups, but other studies have given negative results. In another study of patients hospitalized for cancer and control patients, a greater proportion of artificial sweetener users was found only among women with cancer of the stomach. Little information was available on urinary tract cancer. No overall association was found between artificial sweetener use and cancer.

PROPERTIES

Saccharin is a white crystalline powder with an intensely sweet taste. It is soluble in water, acetone, ethanol, and glycerol and slightly soluble in chloroform and diethyl ether. Saccharin is also available as the calcium and sodium salts. Calcium saccharin is a free-flowing white powder that is odorless or has a faint aromatic odor. Sodium saccharin occurs as white, nondusting granules with no odor. Both salts are soluble in water. When heated to decomposition, saccharin and its calcium and sodium salts emit toxic fumes of nitrogen oxides (NO_x) and sulfur oxides (SO_x). Saccharin is

available as a grade containing up to 98-101% active ingredients. Calcium saccharin is available as a grade 95% pure. Sodium saccharin is available as a grade 98-101% pure.

USE

Saccharin is used primarily as a nonnutritive sweetening agent, with usage increasing substantially after cyclamates were banned in food in 1970. In 1976, the estimated U.S. consumption for all forms of saccharin was 45% in soft drinks; 18% in tabletop sweeteners; 14% in fruit juices, sweets, chewing gum, and jellies; 10% in cosmetics and oral hygiene products; 7% in drugs, such as coatings on pills; 2% in smokeless tobacco products; 2% in electroplating; and 2% for other uses (IARC V.22, 1980).

PRODUCTION

The USITC identified one U.S. producer for saccharin and its sodium salt from 1980 to 1988, but no production data were provided (USITC, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989). The USITC also reported that one U.S. company produced saccharin, calcium salt, from 1982 to 1984, but no production data were provided. U.S. imports of saccharin have steadily declined from 5.9 million lb in 1983 to 3.7 million lb in 1984, about 1.8 million lb in 1985, and to 1.6 million lb in 1987 (USITCa, 1984; USDOC Imports, 1985 1986, 1988). The 1979 TSCA Inventory identified three U.S. companies producing 1.1 million lb of saccharin in 1977, and 6.3 million lb were imported. Two U.S. companies produced 1.6 million lb of saccharin, sodium salt, and 281,000 lb were imported in 1977. Imports of saccharin, calcium salt, amounted to 5,500 lb, and one U.S. company produced 550,000 lb of saccharin, ammonium salt, in 1977 (TSCA, 1979).

EXPOSURE

The primary routes of potential human exposure to saccharin are ingestion and dermal contact. Potential exposure occurs through the consumption of dietetic foods and drinks and some personal hygiene products, such as certain toothpastes and mouthwashes. The FDA has authorized the use of saccharin and its salts in beverages in concentrations not to exceed 12 mg/oz, as a sugar substitute not to exceed 20 mg for each expressed teaspoonful of sugar sweetening equivalency, and in processed food not to exceed 30 mg per serving. In 1983, the Calorie Control Council estimated that in the United States, 44 million adults consumed saccharin-sweetened products. Saccharin consumption is greatest among diabetics and others whose medical conditions require the restriction of calories or carbohydrates. Exposure to saccharin has possibly decreased in recent years due to the introduction of Nutra-Sweet® and Equal® (aspartame). The risk of potential occupational exposure exists for workers involved in the production of saccharin or its salts, in the manufacture and formulation of saccharin-containing products, and during the packaging of the consumer products. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that about 28,000 workers were potentially exposed to saccharin in the workplace (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) estimated that 12,994 total workers, including 11,182 women, potentially were exposed to saccharin and 18,952 total workers, including 11,801 women, potentially were exposed to its sodium salt (NIOSH, 1984). The Toxic Chemical Release Inventory (EPA) listed four industrial facilities that produced, processed or otherwise used saccharin in 1988 (TRI, 1990). In compliance with Community Right-to-Know Program, the facilities reported releases of saccharin to the environment which were estimated to total 750 lb.

REGULATIONS

The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). Saccharin is subject to reporting and recordkeeping rules under CERCLA, RCRA, and SARA. The EPA proposed raising the statutory reportable quantity (RQ) of 1 lb, established under CERCLA, to 100 lb for saccharin and its salts. The final rule adjusts the RQ from 1 lb to 100 lb. Saccharin is regulated as a hazardous constituent of waste under RCRA, and threshold amounts for facilities which may release saccharin have been established under SARA. The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA) as a food ingredient not to exceed specific concentrations. In compliance with the Delaney Clause, the FDA proposed to ban saccharin as a food additive in 1977 because of the available evidence of its carcinogenicity in animals. However, final regulations are pending because of congressional action in 1977 requiring further study and labeling of saccharin. OSHA regulates saccharin under the Hazard Communication Standard and as a chemical hazard in laboratories.