NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for SACCHARIN

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Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

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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.

Summary References

Allen, M. J., E. Boyland, C. E. Dukes, E. S. Horning, and J. G. Watson. 1957. Cancer of the Urinary Bladder Induced in Mice with Metabolites of Aromatic Amines and Tryptophan. Br. J. Cancer 11:212-231.

Althoff, J., A. Cardesa, P. Pour, and P. Shubik. 1975. A Chronic Study of Artificial Sweeteners in Syrian Golden Hamsters. Cancer Lett. 1:21-24.

Armstrong, B., and R. Doll. 1975. Bladder Cancer Mortality in Diabetics in Relation to Saccharin Consumption and Smoking Habits. Br. J. Prev. Soc. Med. 29:73-81.

Arnold, D. L., C. A. Moodie, H. C. Grice, S. M. Charbonneau, B. Stavric, B. T. Collins, P. F. Mcguire, Z. Z. Zawidzka, and I. C. Munro. 1980. Long-Term Toxicity of *ortho*-Toluenesulfonamide and Sodium Saccharin in the Rat. Toxicol. Appl. Pharmacol. 52:113-152.

Ashby, J. 1985. The Genotoxicity of Sodium Saccharin and Sodium Chloride in Relation to Their Cancer-Promoting Properties. Food Chem. Toxicol. 23:507-519.

Batzinger, R. P., S.-Y. L. Ou, and E. Bueding. 1977. Saccharin and Other Sweeteners: Mutagenic Properties. Science 198:944-946.

Bryan, G. T., E. Erturk, and O. Yoshida. 1970. Production of Urinary Bladder Carcinomas in Mice by Sodium Saccharin. Science 168:1238-1240.

Cartwright, R. A., R. Adib, R. Glashan, and B. K. Gray. 1981. The Epidemiology of Bladder Cancer in West Yorkshire. A Preliminary Report on Non-Occupational Aetiologies. Carcinogenesis 2:343-346.

Chowaniec, J., and R. M. Hicks. 1979. Response of the Rat to Saccharin with Particular Reference to the Urinary Bladder. Br. J. Cancer 39:355-375.

Cohen, S. M., M. Arai, J. B. Jacobs, and G. H. Friedell. 1979. Promoting Effect of Saccharin and DL-Tryptophan in Urinary Bladder Carcinogenesis. Cancer Res. 39:1207-1217.

Cohen, S. M., L. L. Arnold, M. Cano, U. Thorgeirsson, and S. Takayama. 1996. Lack of Effect of Sodium Saccharin Feeding on Monkey Urine and Urinary Bladder Epithelium. Proc. Am. Assoc. Cancer Res. 37:108. Abstract.

Fitzhugh, O. G, A. A. Nelson, and J. P. Frawley. 1951. A Comparison of the Chronic Toxicities of Synthetic Sweetening Agents. J. Am. Pharm. Assoc. 40:583-586.

Frederick, C. B., K. L. Dooley, R. L. Kodell, W. G. Sheldon, and F. F. Kadlubar. 1989. The Effect of Lifetime Sodium Saccharin Dosing on Mice Initiated with the Carcinogen 2-Acetylaminofluorene. Fund. Appl. Toxicol. 12:346-357.

Fukushima, S., M. Arai, J. Nakanowatari, T. Hibino, M. Okuda, and N. Ito. 1983. Differences in Susceptibility to Sodium Saccharin Among Various Strains of Rats and Other Animal Species. Gann 74:8-20.

Fukushima, S., S. Uwagawa, T. Shirai, R. Hasegawa, and K. Ogawa. 1990. Synergism by Sodium L-Ascorbate But Inhibition by L-Ascorbic Acid for Sodium Saccharin Promotion of Rat Two-Stage Bladder Carcinogenesis. Cancer Res. 50:4195-4198.

Garland, E. M., J. M. Parr, D. S. Williamson, and S. M. Cohen. 1989a. *In Vitro* Cytotoxicity of the Sodium, Potassium, and Calcium Salts of Saccharin, Sodium Ascorbate, Sodium Citrate, and Sodium Chloride. Toxicol. In Vitro 3:201-205.

Hicks, R. M., and J. Chowaniec. 1977. The Importance of Synergy Between Weak Carcinogens in the Induction of Bladder Cancer in Experimental Animals and Humans. Cancer Res. 37:2943-2949.

Homburger, F. 1978. Negative Lifetime Carcinogen Studies in Rats and Mice Fed 50,000 ppm Saccharin. Chemical Toxicology of Food. Galli, C. L., R. Paoletti, and G. Vettorazzi, Eds. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 359-373.

Hooson, J., R. M. Hicks, P. Grasso, and J. Chowaniec. 1980. *ortho*-Toluene Sulphonamide and Saccharin in the Promotion of Bladder Cancer in the Rat. Br. J. Cancer 42:129-147.

Hoover, R. N., and P. H. Strasser. 1980. Artificial Sweeteners and Human Bladder Cancer: Preliminary Results. Lancet i:837-840.

Howe, G. R., J. D. Burch, A. B. Miller, G. M. Cook, J. Esteve, B. Morrison, P. Gordon, L. W. Chambers, G. Fodor, and G. M. Winsor. 1980. Tobacco Use, Occupation, Coffee, Various Nutrients, and Bladder Cancer. J. Natl. Cancer Inst. 64:701-713.

IARC (International Agency for Research on Cancer). 1980. Saccharin. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 22(Some Non-Nutritive Sweetening Agents):111-170.

IARC (International Agency for Research on Cancer). 1987a. Saccharin. IARC Monogr. Eval. Carcinog. Risks Hum. Suppl. 6(Genetic and Related Effects: An Updating of Selected IARC Monographs From Volumes 1-42):488-496.

IARC (International Agency for Research on Cancer). 1987b. Saccharin. IARC Monogr. Eval. Carcinog. Risks Hum. Suppl. 7(Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1-42):334-339.

JECFA. 1993. The Forty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Series 32. Toxicological Evaluation of Certain Food Additives and Contaminants: Saccharin and Its Salts. International Programme on Chemical Safety (IPCS). World Health Organization, pp. 106-133.

Kroes, R., P. W. J. Peters, J. M. Berkvens, H. G. Verschuuren, T. De Vries, and G. J. van Esch. 1977. Long Term Toxicity and Reproduction Study (Including a Teratogenicity Study) with Cyclamate, Saccharin and Cyclohexylamine. Toxicology 8:285-300.

Lessel, B. 1971. Carcinogenic and Teratogenic Aspects of Saccharin. In: SOS/70 Proceedings of the Third International Congress of Food Science and Technology, Washington, DC, pp. 764-770.

McChesney, E. W., F. Coulston, and K.-F. Benitz. 1977. Six-Year Study of Saccharin in Rhesus Monkeys (Abstract No. 79). Toxicol. Appl. Pharmacol. 41:164. Abstract. (Cited by IARC, 1980)

Mommsen, S., J. Aagaard and A. Sell. 1983. A Case-control Study of Female Bladder Cancer. J. Cancer Clin. Oncol. 19:725-729.

Morrison, A., and J. Buring. 1980. Artificial sweeteners and cancer of the lower urinary tract. N. Engl. J. Med. 302(10):537-541.

Morrison, A. S., W. G. Verhoek, I. Leck, K. Aoki, Y. Ohno, and K. Obata. 1982. Artificial Sweeteners and Bladder Cancer in Manchester, U.K. and Nagoya, Japan. Br. J. Cancer 45:332-336.

Nakanishi, K., M. Hirose, T. Ogiso, R. Hasegawa, M. Arai, and N. Ito. 1980b. Effects of Sodium Saccharin and Caffeine on the Urinary Bladder of Rats Treated with *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine. Gann 71:490-500.

Prasad, O., and G. Rai. 1986. Induction of Papillary Adenocarcinoma of Thyroid in Albino Mice by Saccharin Feeding. Indian J. Exp. Biol. 24:197-199.

Roe, F. J. C., L. S. Levy, and R. L. Carter. 1970. Feeding Studies on Sodium Cyclamate, Saccharin and Sucrose For Carcinogenic and Tumor-Promoting Activity. Food Cosmet. Toxicol. 8:135-145.

Schmähl, D. 1973. Lack of Carcinogenic Effect of Cyclamate, Cyclohexylamine and Saccharin in Rats (German). Arzneim. Forsch. 23:1466-1470. (Cited by IARC, 1980)

Schmähl, D., and M. Habs. 1984. Investigations on the Carcinogenicity of the Artificial Sweeteners Sodium Cyclamate and Sodium Saccharin in Rats in a Two-Generation Experiment. Arzneim. Forsch. 34:604-608.

Schoenig, G. P., E. I. Goldenthal, R. G. Geil, C. H. Frith, W. R. Richter, and F. W. Carlborg. 1985. Evaluation of the Dose Response and *In Utero* Exposure to Saccharin in the Rat. Food Chem. Toxicol. 23:475-490.

Sieber, S. M., and R. H. Adamson. 1978. Long-Term Studies on the Potential Carcinogenicity of Artificial Sweeteners in Non-Human Primates. In: Health and Sugar Substitutes. Guggenheim, B., Ed. Basel, Karger, pp. 266-271. (Cited by IARC, 1980)

Sweatman, T. W., and A. G. Renwick. 1979. Saccharin Metabolism and Tumorigenicity. Science 205:1019-1020.

Sweatman, T. W., and A. G. Renwick. 1980. The Tissue Distribution and Pharmacokinetics of Saccharin in the Rat. Toxicol. Appl. Pharmacol. 5:18-31.

Taylor, J. M., M. A. Weinberger, and L. Friedman. 1980. Chronic Toxicity and Carcinogenicity to the Urinary Bladder of Sodium Saccharin in the in Utero-Exposed Rat. Toxicol. Appl. Pharmacol. 54:57-75.

Thorgeirsson, U., D. Dalgard, J. Reeves, and R. Adamson. 1994. Tumor Incidence in a Chemical Carcinogenesis Study of Nonhuman Primates. Regul. Toxicol. Pharmacol. 19:130-151.

Tisdel, M. O., P. O. Nees, D. L. Harris, and P. H. Derse. 1974. Long-Term Feeding of Saccharin in Rats. In: Symposium: Sweeteners. Inglett, G. E., Ed. Avi Publishing Co., Westport, CN, pp. 145-158.

West, R. W., W. G. Sheldom, D. W. Gaylor, M. G. Haskin, R. R. Delongchamp, and F. F. Kadlubar. 1986. The Effects of Saccharin on the Development of Neoplastic Lesions Initiated with *N*-Methyl-*N*-nitrosourea in the Rat Urothelium. Fundam. Appl. Toxicol. 7:585-600.

Whysner, J., and G. M. Williams. 1996. Saccharin Mechanistic Data and Risk Assessment: Urine Composition, Enhanced Cell Proliferation, and Tumor Promotion. Pharmacol. Ther. 71:225-252.

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

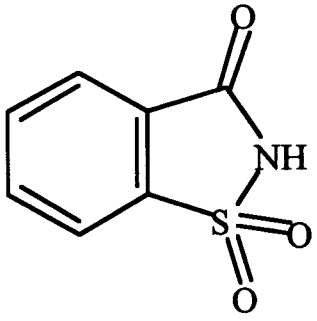
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to 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.)	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	Relative Risks for Use of Artificial Sweeteners in Subgroups; no. cases/controls: 1.3; 14/34 1.8; 13/22 2.7; 16/18 all RR had p < 0.01; 95% CI not provided	age			Hoover and Strasser (1980)
	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 sweet-ener) ≥ 2 cans diet beverage/d ay (≥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 occasion-ally 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

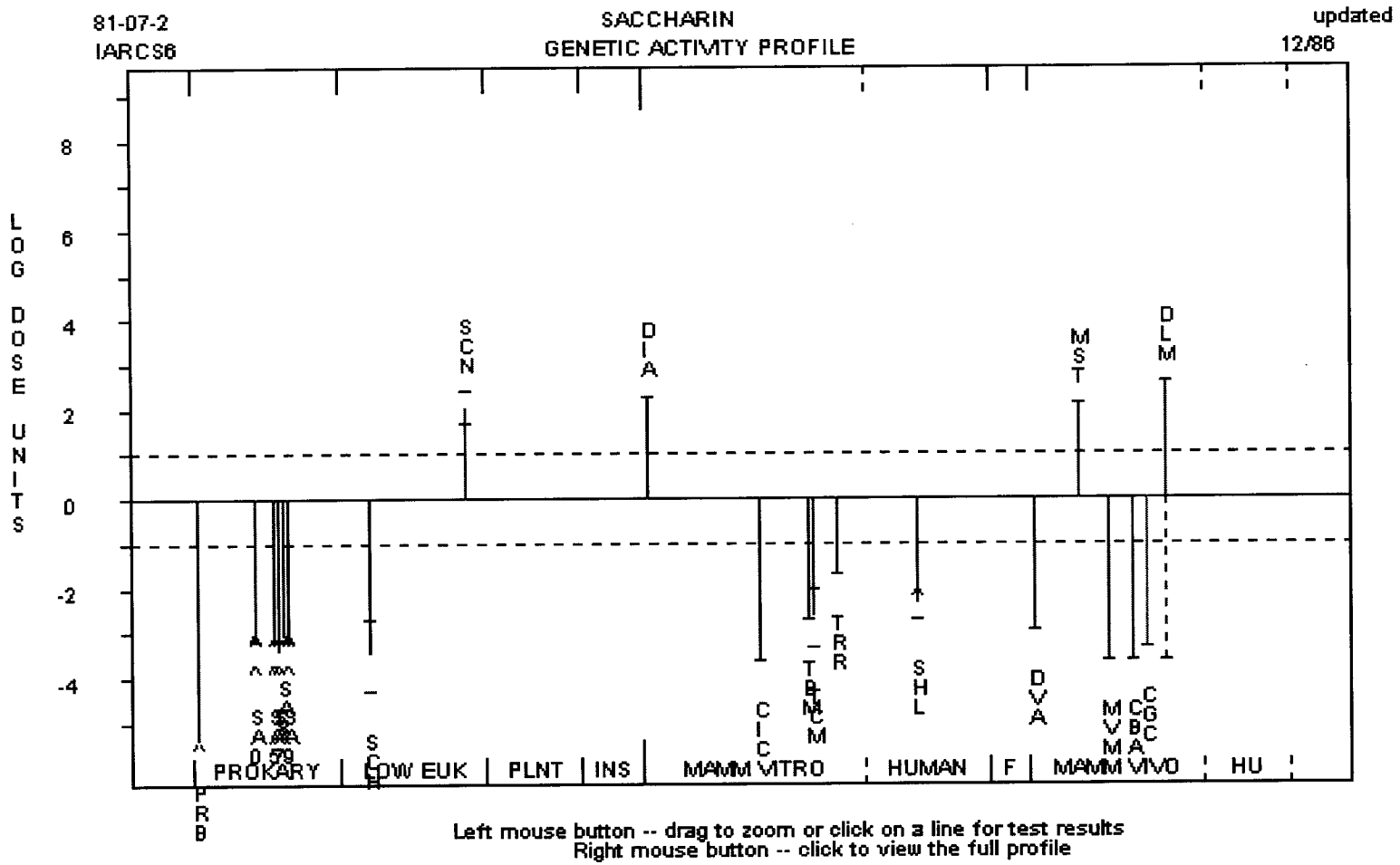


Figure 5-2. Genetic Activity Profile (GAP) for Sodium Saccharin

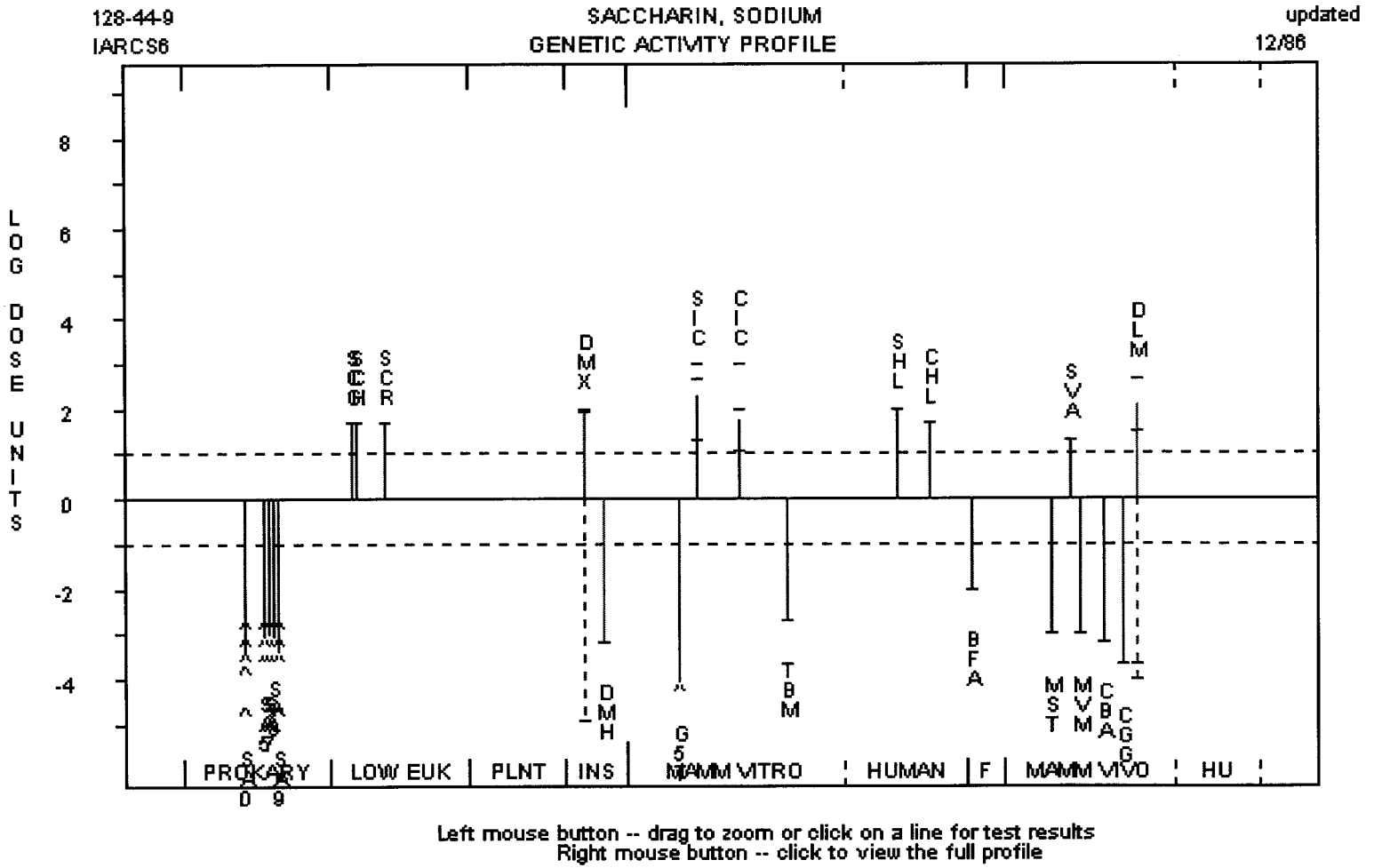
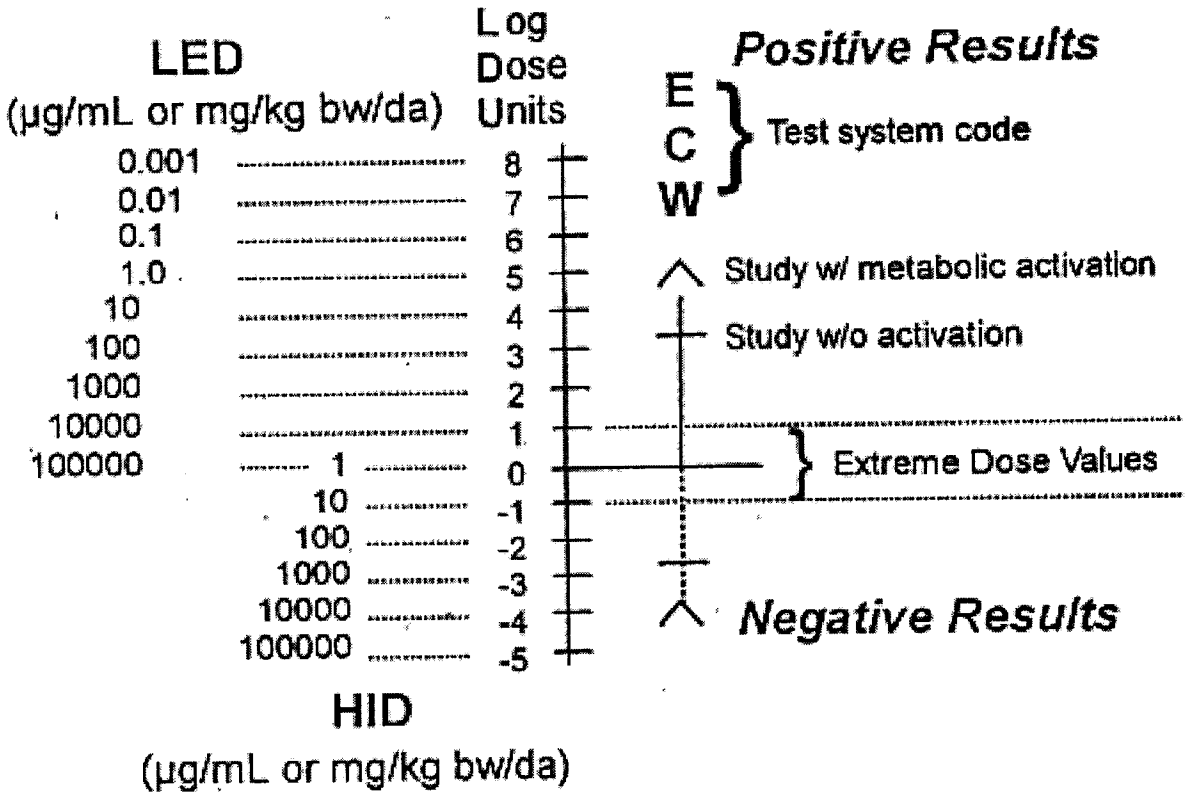


Figure 5-3. Schematic View of a Genetic Activity Profile



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 $\alpha 2\mu$-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 Carcinoma	
	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)

8.0 REFERENCES

Adler, I., and J. Ashby. 1989. The Present Lack of Evidence for Unique Rodent Germ-Cell Mutagens. Mutat. Res. 212:55-66.

Allen, M. J., E. Boyland, C. E. Dukes, E. S. Horning, and J. G. Watson. 1957. Cancer of the Urinary Bladder Induced in Mice with Metabolites of Aromatic Amines and Tryptophan. Br. J. Cancer 11:212-231.

Althoff, J., A. Cardesa, P. Pour, and P. Shubik. 1975. A Chronic Study of Artificial Sweeteners in Syrian Golden Hamsters. Cancer Lett. 1:21-24.

Anderson, R. L. 1988. An Hypothesis of the Mechanism of Urinary Bladder Tumorigenesis in Rats Ingesting Sodium Saccharin. Food Chem. Toxicol. 26:637-644.

Anderson, R. L., W. R. Francis and F. R. Lefever. 1987a. Effect of Dietary Carbohydrate Type and Content on the Response of Male Rats to Dietary Sodium Saccharin. Food Chem. Toxicol. 25:271-275.

Anderson, R. L., F. R. Lefever, and J. K. Maurer. 1987b. Effect of Inherent Urine Output of the Response of Male Rats to 7.5% Dietary Sodium Saccharin. Food Chem. Toxicol. 25:641-645.

Anderson, R. L., F. R. Lefever, and J. K. Maurer. 1988. The Effect of Various Saccharin Forms on Gastro-Intestinal Tract, Urine and Bladder of Male Rats. Food Chem. Toxicol. 26:665-669.

Armstrong, B., and R. Doll. 1974. Bladder Cancer Mortality in England and Wales in Relation to Cigarette Smoking and Saccharin Consumption. Br. J. Prev. Soc. Med. 28:233-240.

Armstrong, B., and R. Doll. 1975. Bladder Cancer Mortality in Diabetics in Relation to Saccharin Consumption and Smoking Habits. Br. J. Prev. Soc. Med. 29:73-81.

Arnold, D. L. 1983. Two-Generation Saccharin Bioassays. Environ. Health Perspect. 50:27-36.

Arnold, D. L., C. A. Moodie, H. C. Grice, S. M. Charbonneau, B. Stavric, B. T. Collins, P. F. Mcguire, Z. Z. Zawidzka, and I. C. Munro. 1980. Long-Term Toxicity of *ortho*-Toluenesulfonamide and Sodium Saccharin in the Rat. Toxicol. Appl. Pharmacol. 52:113-152.

Arnold, D. L., D. Krewski, and I. C. Munro. 1983. Saccharin: A Toxicological and Historical Perspective. Toxicology 27:179-256.

Ashby, J. 1985. The Genotoxicity of Sodium Saccharin and Sodium Chloride in Relation to Their Cancer-Promoting Properties. Food Chem. Toxicol. 23:507-519.

Ball, L. M., A. G. Renwick, and R. T. Williams. 1977. The Fate of [^{14}C]Saccharin in Man, Rat, and Rabbit and of 2-Sulphamoyl [^{14}C]Benzoic Acid in the Rat. Xenobiotica 7:189-203.

Batzinger, R. P., S.-Y. L. Ou, and E. Bueding. 1977. Saccharin and Other Sweeteners: Mutagenic Properties. Science 198:944-946.

Beljanski, M., L. Le Goff, and M. Beljanski. 1982. *In Vitro* Screening of Carcinogens Using DNA of the His$^-$ Mutant of *Salmonella typhimurium*. Exp. Cell Biol. 50:271-280.

Bryan, G. T., E. Erturk, and O. Yoshida. 1970. Production of Urinary Bladder Carcinomas in Mice by Sodium Saccharin. Science 168:1238-1240.

Budavari, S., Ed. 1996. The Merck Index, 12th ed. Merck & Co., Inc., Whitehall, NJ.

Burin, G., H. Gibb, and R. Hill. 1995a. Human Bladder Cancer: Evidence for a Potential Irritation-Induced Mechanism. Food Chem. Toxicol. 33:785-795.

Burin, G. J., D. B. Clayson, S. M. Cohen, J. M. DeSesso, L. B. Ellwein, L. Fishbein, C. Frederick, H. Gibb, N. J. Gorelick, G. C. Hard, C. King, R. J. Lorentzen, R. Oyasu, J. M. Rice, C. Y. Wang, and J. M. Ward. 1995b. Urinary Bladder Carcinogenesis: Implications for Risk Assessment. Food Chem. Toxicol. 33:797-802.

Burns, P. C., and E. Rozengurt. 1984. Extracellular Na^+ and Initiation of DNA Synthesis: Role on Intracellular pH and K^+. J. Cell Biol. 98:1082-1089.

Byard, J. L., and L. Golberg. 1973. The Metabolism of Saccharin in Laboratory Animals. Food Cosmet. Toxicol. 11:391-402.

Byard, J. L., E. W. McChesney, L. Goldberg, and F. Coulston. 1974. Excretion and Metabolism of Saccharin in Man. II. Studies with ^{14}C-Labelled and Unlabelled Saccharin. Food Cosmet. Toxicol. 12:175-184.

Calorie Control Council. 1996. Saccharin: A Scientific Review. Petition to Delist Saccharin From National Toxicology Program's Report on Carcinogens. 143 pp.

Cameron, I. L., N. K. R. Smith, T. B. Pool, and R. L. Sparks. 1980. Intracellular Concentrations of Sodium and Other Elements as Related to Mitogenesis and Oncogenesis *In Vivo*. Cancer Res. 40:1493-1500.

Cartwright, R. A., R. Adib, R. Glashan, and B. K. Gray. 1981. The Epidemiology of Bladder Cancer in West Yorkshire. A Preliminary Report on Non-Occupational Aetiologies. Carcinogenesis 2:343-346.

Chappel, C. I. 1992. A Review and Biological Risk Assessment of Sodium Saccharin. Regul. Toxicol. Pharmacol. 15:253-270.

Chowaniec, J., and R. M. Hicks. 1979. Response of the Rat to Saccharin with Particular Reference to the Urinary Bladder. Br. J. Cancer 39:355-375.

Clayson, D. B., L. Fishbein, and S. M. Cohen. 1995. Effects of Stones and Other Physical Factors on the Induction of Rodent Bladder Cancer. Food Chem. Toxicol. 33:771-784.

Cohen, S. 1994a. Slide 11: Effect of Various Forms of Saccharin on the Rat Urinary Bladder. In: Transcripts from the Workshop, Assessing the Cancer Risk of Saccharin and Sodium Saccharin. San Francisco, CA, April 14-16. Sponsored by California EPA.

Cohen, S. 1994b. Slide 12: Urine Analysis in Rats Given Various Forms of Saccharin for 4 Weeks. In: Transcripts from the Workshop, Assessing the Cancer Risk of Saccharin and Sodium Saccharin. San Francisco, CA, April 14-16. Sponsored by California EPA.

Cohen, S. 1994c. Slide 27: Interspecies Comparison of Sodium Saccharin. In: Transcripts from the Workshop, Assessing the Cancer Risk of Saccharin and Sodium Saccharin. San Francisco, CA, April 14-16. Sponsored by California EPA.

Cohen, S. 1994d. Slide 28: Fresh Void Urine Chemistries. In: Transcripts from the Workshop, Assessing the Cancer Risk of Saccharin and Sodium Saccharin. San Francisco, CA, April 14-16. Sponsored by California EPA.

Cohen, S. 1995a. Human Relevance of Animal Carcinogenicity Studies. Regul. Toxicol. Pharmacol. 21:75-80.

Cohen, S. 1995b. Cell Proliferation in the Bladder and Implications for Cancer Risk Assessment. Toxicology 102:149-159.

Cohen, S. 1995c. Role of Urinary Physiology and Chemistry in Bladder Carcinogenesis. Food Chem. Toxicol. 33:715-730.

Cohen, S. M., and L. B. Ellwein. 1991. Cell Proliferation and Bladder Tumor Promotion. Progress in Clinical and Biological Research, Vol. 369. Chemically Induced Cell Proliferation: Implications for Risk Assessment; Chemically Induced Cell Proliferation Conference, Austin, TX, USA; November 29-December 2, 1989. Butterworth, B. E., et al., Eds. Wiley-Liss, Inc., New York, NY, pp. 347-356.

Cohen, S., and T. Lawson. 1995. Rodent Bladder Tumors Do Not Always Predict for Humans. Cancer Lett. 93:9-16.

Cohen, S. M., M. Arai, J. B. Jacobs, and G. H. Friedell. 1979. Promoting Effect of Saccharin and DL-Tryptophan in Urinary Bladder Carcinogenesis. Cancer Res. 39:1207-1217.

Cohen, S. M., G. Murasaki, S. Fukushima, and R.E. Greenfield. 1982. Effect of Regenerative Hyperplasia on the Urinary Bladder: Carcinogenicity of Sodium Saccharin and *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 42:65-71.

Cohen, S. M., M. Cano, E. M. Garland, and R. A. Earl. 1989. Silicate Crystals in the Urine and Bladder Epithelium of Male Rats Fed Sodium Saccharin. Carcinogenesis 30:A205. Abstract.

Cohen, S., M. Fisher, T. Sakata, M. Cano, G. Schoenig, C. Chappel, and E. Garland. 1990. Comparative Analysis of the Proliferative Response of the Rat Urinary Bladder to Sodium Saccharin by Light and Scanning Electron Microscopy and Autoradiography. Scanning Microsc. 4:135-142.

Cohen, S., M. Cano, R. Earl, S. Carson, and E. Garland. 1991a. A Proposed Role for Silicates and Protein in the Proliferative Effects of Saccharin on the Male Rat Urothelium. Carcinogenesis 12:1551-1555.

Cohen, S., L. Ellwein, T. Okamura, T. Masui, S. Johansson, R. Smith, J. Wehner, M. Khachab, C. Chappel, G. Schoenig, J. Emerson, and E. Garland. 1991b. Comparative Bladder Tumor Promoting Activity of Sodium Saccharin, Sodium Ascorbate, Related Acids, and Calcium Salts in Rats. Cancer Res. 51:1766-1777.

Cohen, S. M., M. Cano, E. M. Garland, M. St. John, and L. L. Arnold. 1995a. Urinary and Urothelial Effects of Sodium Salts in Male Rats. Carcinogenesis 16:343-348.

Cohen, S. M., M. Cano, M. K. St. John, E. M., Garland, M. Khachab, and L. B. Ellwein. 1995b. Effect of Sodium Saccharin on the Neonatal Rat Bladder. Scanning Microsc. 9:137-148.

Cohen, S. M., E. M. Garland, M. Cano, M. St. John, M. Khachab, J. M. Wehner, and L. L. Arnold. 1995c. Effects of Sodium Ascorbate, Sodium Saccharin and Ammonium Chloride on the Male Rat Urinary Bladder. Carcinogenesis 16:2743-2750.

Cohen, S. M., L. L. Arnold, M. Cano, U. Thorgeirsson, and S. Takayama. 1996. Lack of Effect of Sodium Saccharin Feeding on Monkey Urine and Urinary Bladder Epithelium. Proc. Am. Assoc. Cancer Res. 37:108. Abstract.

Cohen, S. M., T. Masui, E. M. Garland, and L. L. Arnold. 1997. Effects of Diet on Urinary Bladder Carcinogenesis and Cancer Prevention. J. Nutr. 127(Suppl. 5):826S-829S.

Connolly, J. G., W. D. Rider, L. Rosenbaum, and J.-A. Chapman. 1978. Relation Between the Use of Artificial Sweeteners and Bladder Cancer. Can. Med. Assoc. J. 119:408.

Crammer, B., and R. Ikan. 1977. Properties and Syntheses of Sweetening Agents. Chem. Soc. Rev. 6:431-453. (Cited by IARC, 1980)

Cranmer, M. F. 1980. Saccharin: A Report. [Scherr, G. H., Ed.] Pathotox Publishers, Park Forest South, IL. 586 pp.

Dawson, H. 1994a. Economic Substitution. Beverage World 55-58, April.

Dawson, H. 1994b. Holding Aces. Beverage World 58-60, April.

Debiec-Rychter, M., and C. Y. Wang. 1990. Induction of DNA Synthesis by Sodium Phenobarbital, Uracil, and Sodium Saccharin in Urinary Bladder of the F344 Rat. Toxicol. Appl. Pharmacol. 105:345-349.

DeFlora, S., P. Zanacchi, A. Camoirano, C. Bennicelli, and G. S. Badolati. 1984. Genotoxic Activity and Potency of 135 Compounds in the Ames Reversion Test and in a Bacterial DNA-Repair Test. Mutat. Res. 133:161-198.

DeSesso, J. 1995. Anatomical Relationships of Urinary Bladders Compared: Their Potential Role in the Development of Bladder Tumours in Humans and Rats. Food Chem. Toxicol. 33:705-714.

Dropkin, R. H., D. F. Salo, S. M. Tucci, and G. I. Kaye. 1985. Effects on Mouse Embryos of *In Utero* Exposure to Saccharin: Teratogenic and Chromosome Effects. Arch. Toxicol. 56:283-287.

Elcock, M., and R. Morgan. 1993. Update on Artificial Sweeteners and Bladder Cancer. Regul. Toxicol. Pharmacol. 17:35-43.

Ellwein, L. B., and S. M. Cohen. 1988. A Cellular Dynamics Model of Experimental Bladder Cancer: Analysis of the Effect of Sodium Saccharin in the Rat. Risk Anal. 8:215.

Ellwein, L., and S. Cohen. 1990. The Health Risks of Saccharin Revisited. Crit. Rev. Toxicol. 20:311-326.

Ershoff, B. H., and G.S. Bajwa. 1974. Inhibitory Effect of Sodium Cyclamate and Sodium Saccharin on Tumor Induction by 2-Acetylaminofluorene in Rats. Proc. Soc. Exp. Biol. (NY) 145:1293-1297. (Cited by IARC, 1980)

Fisher, M. J., T. Sakata, T. S. Tibbels, R. A. Smith, K. Patil, M. Khachab, S. L. Johansson, and S. M. Cohen. 1989. Effect of Sodium Saccharin and Calcium Saccharin on Urinary Parameters in Rats Fed Prolab 3200 or AIN-76 Diet. Food Chem. Toxicol. 27:1-9.

Fitzhugh, O. G, A. A. Nelson, and J. P. Frawley. 1951. A Comparison of the Chronic Toxicities of Synthetic Sweetening Agents. J. Am. Pharm. Assoc. 40:583-586.

Frederick, C. B., K. L. Dooley, R. L. Kodell, W. G. Sheldon, and F. F. Kadlubar. 1989. The Effect of Lifetime Sodium Saccharin Dosing on Mice Initiated with the Carcinogen 2-Acetylaminofluorene. Fund. Appl. Toxicol. 12:346-357.

Fukushima, S., and S. M. Cohen. 1980. Saccharin-Induced Hyperplasia of the Rat Urinary Bladder. Cancer Res. 40:734-736.

Fukushima, S., G. H. Friedell, J. B. Jacobs, and S. M. Cohen. 1981. Effect of L-Tryptophan and Sodium Saccharin on Urinary Tract Carcinogenesis Initiated by *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 41:3100-3103.

Fukushima, S., M. Arai, J. Nakanowatari, T. Hibino, M. Okuda, and N. Ito. 1983. Differences in Susceptibility to Sodium Saccharin Among Various Strains of Rats and Other Animal Species. Gann 74:8-20.

Fukushima, S., M. Shibata, T. Shirai, S. Tamano, and N. Ito. 1986. Roles of Urinary Sodium Ion Concentration and pH in Promotion by Ascorbic Acid of Urinary Bladder Carcinogenesis in Rats. Cancer Res. 46:1623-1626.

Fukushima, S., S. Tamano, M. A. Shibata, Y. Kurata, M. Hirose, and N. Ito. 1988. The Role of Urinary pH and Sodium Ion Concentration in the Promotion Stage of Two-stage Carcinogenesis of the Rat Urinary Bladder. Carcinogenesis 9:1203-1206.

Fukushima, S., S. Uwagawa, T. Shirai, R. Hasegawa, and K. Ogawa. 1990. Synergism by Sodium L-Ascorbate But Inhibition by L-Ascorbic Acid for Sodium Saccharin Promotion of Rat Two-Stage Bladder Carcinogenesis. Cancer Res. 50:4195-4198.

Furuya, T., K. Kawamata, T. Kaneko, O. Uchida, S. Horiuchi, and Y. Ikeda. 1975. Long-term Toxicity Study of Sodium Cyclamate and Saccharin Sodium in Rats. Jpn. J. Pharmacol. 25:55P-56P. Abstract.

Garland, E. M., J. M. Parr, D. S. Williamson, and S. M. Cohen. 1989a. *In Vitro* Cytotoxicity of the Sodium, Potassium, and Calcium Salts of Saccharin, Sodium Ascorbate, Sodium Citrate, and Sodium Chloride. Toxicol. In Vitro 3:201-205.

Garland, E. M., T. Sakata, M. J. Fisher, M. Tsuneo, and S. M. Cohen. 1989b. Influences of Diet and Strain on the Proliferation Effect on the Rat Urinary Bladder Induced by Sodium Saccharin. Cancer Res. 49:3789-3794.

Garland, E. M., P. L. Kraft, R. Shapiro, M. Khachab, K. Patil, L. B. Ellwein, and S. M. Cohen. 1991. Effects of *In Utero* and Postnatal Sodium Saccharin Exposure on the Nutritional Status of the Young Rat. I. Effects at 30 Days Post-Birth. Food Chem. Toxicol. 29:657-67.

Garland, E., M. St. John, M. Asamoto, S. Eklund, B. Mattson, L. Johnson, M. Cano, and S. Cohen. 1994. Comparison of the Effects of Sodium Saccharin in NBR Rats and in Intact and Castrated Male F344 Rats. Cancer Lett. 78:99-107.

Hard, G. 1995. Species Comparison of the Content and Composition of Urinary Proteins. Food Chem. Toxicol. 33:731-746.

Harguindey, S., J. L. Pedraz, R. G. Cañero, J. Pérez de Diego, and E. J. Cragoe, Jr. 1995. Hydrogen Ion-Dependent Oncogenesis and Parallel New Avenues to Cancer Prevention and Treatment Using a H^+-Mediated Unifying Approach: pH-Related and pH-Unrelated Mechanisms. Crit. Rev. Oncogen. 6:1-33.

Hasegawa, R., and S. M. Cohen. 1986. The Effect of Different Salts of Saccharin on the Rat Urinary Bladder. Cancer Lett. 30:261-268.

Hasegawa, R., M. K. St. John, M. Cano, P. Issenberg, D.A. Klein, B. A. Walker, J. W. Jones, R. C. Schnell, B. A. Merrick, M. H. Davies, D. T. McMillan, and S. M. Cohen. 1984. Bladder Freeze Ulceration and Sodium Saccharin Feeding in the Rat: Examination for Urinary Nitrosamines, Mutagens and Bacteria, and Effects on Hepatic Microsomal Enzymes. Food Chem. Toxicol. 22(12):935-942.

Hasegawa, R., R. E. Greenfield, G. Murasaki, T. Suzuki, and S. M. Cohen. 1985. Initiation of Urinary Bladder Carcinogenesis in Rats by Freeze Ulceration with Sodium Saccharin Promotion. Cancer Res. 45:1469-1473.

Heil, J., and G. Reifferscheid. 1992. Detection of Mammalian Carcinogens with an Immunological DNA Synthesis-Inhibition Test. Carcinogenesis 12:2389-94.

Hicks, R. M. 1983. Effect of Promoters on Incidence of Bladder Cancer in Experimental Animal Models. Environ. Health Perspect. 50:37-49.

Hicks, R. M. 1984. Promotion: Is Saccharin a Promoter in the Urinary Bladder? Food Chem. Toxicol. 22:755-760.

Hicks, R. M., and J. Chowaniec. 1977. The Importance of Synergy Between Weak Carcinogens in the Induction of Bladder Cancer in Experimental Animals and Humans. Cancer Res. 37:2943-2949.

Hicks, R. M., J. Wakefield, and J. Chowaniec. 1973. Co-carcinogenic Action of Saccharin in the Chemical Induction of Bladder Cancer. Nature 243:347-349.

Hicks, R. M., J. S. J. Wakefield, and J. Chowaniec. 1975. Evaluation of a New Model to Detect Bladder Carcinogens or Co-Carcinogens; Results Obtained with Saccharin, Cyclamate and Cyclophosphamide. Chem. Biol. Interact. 11:225-233.

Hicks, R. M., J. Chowaniec, and J. St. J. Wakefield. 1978. Experimental Induction of Bladder Tumors By a Two-Stage System. In: Carcinogenesis, Vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis. Slaga, T. J., A. Sivak, and R. K. Boutwell, Eds. Raven Press, NY, pp. 475-489.

Homburger, F. 1978. Negative Lifetime Carcinogen Studies in Rats and Mice Fed 50,000 ppm Saccharin. Chemical Toxicology of Food. Galli, C. L., R. Paoletti, and G. Vettorazzi, Eds. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 359-373.

Homma, Y., Y. Kondo, T. Kakizoe, Y. Aso, and S. Nagase. 1991. Lack of Bladder Carcinogenicity of Dietary Sodium Saccharin in Analbuminaemic Rats, Which Are Highly Susceptible to *N*-Nitroso-*n*-butyl-(4-hydroxybutyl)amine. Food Chem. Toxicol. 29:373-376.

Hooson, J., R. M. Hicks, P. Grasso, and J. Chowaniec. 1980. *ortho*-Toluene Sulphonamide and Saccharin in the Promotion of Bladder Cancer in the Rat. Br. J. Cancer 42:129-147.

Hoover, R. N., and P. H. Strasser. 1980. Artificial Sweeteners and Human Bladder Cancer: Preliminary Results. Lancet i:837-840.

Hoover, R., and P. Hartge. 1982. Non-Nutritive Sweeteners and Bladder Cancer. Am. J. Public Health 72:382-383. (Cited by IARC, 1987b)

Howe, G. R., J. D. Burch, A. B. Miller, B. Morrison, P. Gordon, L. Weldon, L. W. Chambers, G. Fodor, and G. M. Winsor. 1977. Artificial Sweeteners and Human Bladder Cancer. Lancet ii:578-581.

Howe, G. R., J. D. Burch, A. B. Miller, G. M. Cook, J. Esteve, B. Morrison, P. Gordon, L. W. Chambers, G. Fodor, and G. M. Winsor. 1980. Tobacco Use, Occupation, Coffee, Various Nutrients, and Bladder Cancer. J. Natl. Cancer Inst. 64:701-713.

HSDB (Hazardous Substances Data Bank). 1996. Saccharin. Online available from the National Library of Medicine's TOXNET system. Profile last updated 1/19/96.

IARC (International Agency for Research on Cancer). 1980. Saccharin. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 22(Some Non-Nutritive Sweetening Agents):111-170.

IARC (International Agency for Research on Cancer). 1982. Saccharin. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. Suppl. 4(Chemicals, Industrial Processes and Industries Associated with Cancer in Humans):224-226.

IARC (International Agency for Research on Cancer). 1987a. Saccharin. IARC Monogr. Eval. Carcinog. Risks Hum. Suppl. 6(Genetic and Related Effects: An Updating of Selected IARC Monographs From Volumes 1-42):488-496.

IARC (International Agency for Research on Cancer). 1987b. Saccharin. IARC Monogr. Eval. Carcinog. Risks Hum. Suppl. 7(Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1-42):334-339.

Imaida, K., and C.Y. Wang. 1986. Effect of Sodium Phenobarbital and Sodium Saccharin in AIN-76A Diet on Carcinogenesis Initiated with *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide and *N,N*-Dibutylnitrosamine in Male F344 Rats. Cancer Res. 46:6160-6164.

Irving-Monshaw, S. 1989. New Sugar Substitutes Are Poised to Hit the Table. Chem. Eng. News, pp. 47-50, July.

Iscovich, J., R. Castelletto, J. Estève, N. Muñoz, R. Colanzi, A. Coronel, I. Deamezola, V. Tassi, and A. Arslan. 1987. Tobacco Smoking, Occupational Exposure and Bladder Cancer in Argentina. Int. J. Cancer 40:734-740.

Ito, N., and S. Fukushima. 1989. Promotion of Urinary Bladder Carcinogenesis in Experimental Animals. Exp. Pathol. 36:1-15.

JECFA. 1993. The Forty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Series 32. Toxicological Evaluation of Certain Food Additives and Contaminants: Saccharin and Its Salts. International Programme on Chemical Safety (IPCS). World Health Organization, pp. 106-133.

Jensen, O. M., and C. Kamby. 1982. Intra-uterine Exposure to Saccharin and Risk of Bladder Cancer in Man. Int. J. Cancer 29:507-509.

Kennedy, G., O. E. Fancher, J. C. Calandra, and R. E. Keller. 1972. Metabolic Fate of Saccharin in the Albino Rat. Food Cosmet. Toxicol. 10:143-149.

Kessler, I. I., and J. P. Clark. 1978. Saccharin, Cyclamate, and Human Bladder Cancer. No Evidence of an Association. J. Am. Med. Assoc. 240:349-355. (Cited by IARC, 1980)

Klaunig, J. E., and R. J. Ruch. 1990. Role of Inhibition of Intercellular Communication in Carcinogenesis. Lab. Invest. 62:135-146.

Kroes, R., P. W. J. Peters, J. M. Berkvens, H. G. Verschuuren, T. De Vries, and G. J. van Esch. 1977. Long Term Toxicity and Reproduction Study (Including a Teratogenicity Study) with Cyclamate, Saccharin and Cyclohexylamine. Toxicology 8:285-300.

Lawson, T. A., and P. J. Hertzog. 1981. The Failure of Chronically Administered Saccharin to Stimulate Bladder Epithelial DNA Synthesis in FO Rats. Cancer Lett. 11:221-224.

Lehman-McKeeman, L. D., and D. Caudill. 1991. Quantitation of Urinary α2μ-Globulin and Albumin in by Reverse-Phase High Performance Liquid Chromatography. J. Pharmacol. Methods 26:239-247.

Lehman-McKeeman, L. D., M. I. Rivera-Torres, and D. Caudill. 1990. Lysosomal Degradation of α2μ-Globulin and α2μ-Globulin-Xenobiotic Conjugates. Toxicol. Appl. Pharmacol. 103:539-548.

Lessel, B. 1970. Carcinogenic and Teratogenic Aspects of Saccharin. In: Proceedings SOS/70 Third International Congress of Food Science and Technology, Washington, DC, pp. 764-770.

Lethco, E. J., and W. C. Wallace. 1975. The Metabolism of Saccharin in Animals. Toxicology 3:287-300.

Lutz, W. K., and C. H. Schlatter. 1977. Saccharin Does Not Bind to DNA of Liver or Bladder in the Rat. Chem. Biol. Interact. 19:253-257.

Ma, T. H., Z. Xu, C. Xu, H. McConnell, E. V. Rabago, G. A. Arreola, and H. Zhang. 1995. The Improved Allium/Vicia Root Tip Micronucleus Assay for Clastogenicity of Environmental Pollutants. Mutat. Res. 334:185-195.

MAFF. 1994. Food Safety Directorate Food Surveillance Information Sheet. Number 46. MAFF U.K. Survey of the Intake of Sweeteners by Diabetics. http://www.maff.gov.uk/food/infsheet/1994/no46/46sweet.htm.

Masui, T., T. Sakata, E.M. Garland, L. B. Ellwein, S. L. Johansson, and S. M. Cohen. 1988. Effects of Sodium Saccharin (NaS) on Rat Fetal and Neonatal Urinary Bladder. Proc. AACR, 29:161. Abstract.

Masui, T., A. M. Mann, T. L. Macatee, T. Okamura, E. M. Garland, H. Fujii, J. C. Pelling, and S. M. Cohen. 1991. H-*ras* Mutations in Rat Urinary Bladder Carcinomas Induced by *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide and Sodium Saccharin, Sodium Ascorbate or Related Salts. Cancer Res. 51:3471-3475

Matthews, H. B., M. Fields, and L. Fishbein. 1973. Saccharin: Distribution and Excretion of a Limited Dose in the Rat. J. Agric. Food Chem. 21:916-919.

McChesney, E. W., F. Coulston, and K.-F. Benitz. 1977. Six-Year Study of Saccharin in Rhesus Monkeys (Abstract No. 79). Toxicol. Appl. Pharmacol. 41:164. Abstract. (Cited by IARC, 1980)

Miller, C. T., C. I. Neutel, R. C. Nair, L. D. Marrett, J. M. Last, and W. E. Collins. 1978. Relative Importance of Risk Factors in Bladder Carcinogenesis. J. Chronic Dis. 31:51-56. (Cited by IARC, 1980)

Mohr, U., U. Green, J. Althoff, and P. Schneider. 1978. Syncarcinogenic Action of Saccharin and Sodium-Cyclamate in the Induction of Bladder Tumours in MNU-Pretreated Rats. In: Health and Sugar Substitutes. Guggenheim, B., Ed. Karger, Basel, pp. 64-69.

Møller-Jensen, O., J. B. Knudsen, B. L. Sørensen, and J. Clemmesen. 1983. Artificial Sweeteners and Absence of Bladder Cancer Risk in Copenhagen. Int. J. Cancer 32:577-582.

Momas, I., J.-P. Daurès, B. Festy, J. Bontoux, and F. Grémy. 1994. Relative Importance of Risk Factors in Bladder Carcinogenesis: Some New Results about Mediterranean Habits. Cancer Causes Controls 5:326-332.

Mommsen, S., J. Aagaard, and A. Sell. 1983. A Case-control Study of Female Bladder Cancer. J. Cancer Clin. Oncol. 19:725-729.

Morgan, R. W., and M. G. Jain. 1974. Bladder Cancer: Smoking, Beverages, and Artificial Sweeteners. Can. Med. Assoc. J. 111:1067-1070.

Morgan, R., and O. Wong. 1985. Review of Epidemiological Studies on Artificial Sweeteners and Bladder Cancer. Food Chem. Toxicol. 23:529-533.

Morrison, A., and J. Buring. 1980. Artificial Sweeteners and Cancer of the Lower Urinary Tract. N. Engl. J. Med. 302(10):537-541.

Morrison, A. S., W. G. Verhoek, I. Leck, K. Aoki, Y. Ohno, and K. Obata. 1982. Artificial Sweeteners and Bladder Cancer in Manchester, U.K. and Nagoya, Japan. Br. J. Cancer 45:332-336.

Munro, I. C., C. A. Noodie, D. Krewski, and H. C. Grice. 1975. A Carcinogenicity Study of Commercial Saccharin in the Rat. Toxicol. Appl. Pharmacol. 32:513-526.

Murai, T., S. Mori, M. Hosomo, A. Takashima, S. Machino, T. Oohara, H. Yamashita, S. Makino, T. Matsuda, H. Wanibuchi, and S. Fukushima. 1997. Strain Differences in Sensitivity to

the Promoting Effect of Sodium L-Ascorbate in a Two-Stage Rat Urinary Bladder Carcinogenesis Model. Jpn. J. Cancer Res. 88:245-253.

Murasaki, G., and S. M. Cohen. 1981. Effect of Dose of Sodium Saccharin on the Induction of Rat Urinary Bladder Proliferation. Cancer Res. 41:942-944.

Murasaki, G., and S. M. Cohen. 1983a. Co-carcinogenicity of Sodium Saccharin and *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide for the Urinary Bladder. Carcinogenesis 4:97-99.

Murasaki, G., and S. M. Cohen. 1983b. Effect of Sodium Saccharin on Urinary Bladder Epithelial Regenerative Hyperplasia Following Freeze Ulceration. Cancer Res. 43(1):182-187.

Najem, G. R., D. B. Louria, J. J. Seebode, I. S. Thind, J. M. Prusakowski, R. B. Ambrose, and A. R. Fernicola. 1982. Life Time Occupation, Smoking, Caffeine, Saccharine, Hair Dyes and Bladder Carcinogenesis. Int. J. Epidemiol. 11:212-217.

Nakanishi, K., A. Hagiwara, M. Shibata, K. Imaida, W. Tatematsu, and N. Ito. 1980a. Dose Response of Saccharin in Induction of Urinary Bladder Hyperplasias in Fischer 344 Rats Pretreated with *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine. J. Natl. Cancer Inst. 65:1005-1010.

Nakanishi, K., M. Hirose, T. Ogiso, R. Hasegawa, M. Arai, and N. Ito. 1980b. Effects of Sodium Saccharin and Caffeine on the Urinary Bladder of Rats Treated with *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine. Gann 71:490-500.

Nakanishi, K., S. Fukushima, A. Hagiwara, S. Tamano, and N. Ito. 1982. Organ-Specific Promoting Effects of Phenobarbital Sodium and Sodium Saccharin in the Induction of Liver and Urinary Bladder Tumors in Male F344 Rats. J. Natl. Cancer Inst. 68:497-500.

National Academy of Sciences-National Research Council. 1974. Report to FDA on the Safety of Saccharin and Sodium Saccharin in the Human Diet. Publication No. 238 137. (Cited by Arnold et al., 1980)

Neuhaus, O., and W. Flory. 1978. Age-Dependent Changes in the Excretion of Urinary Proteins by the Rat. Nephron 22:570-576.

NIOSH (National Institute of Occupational Safety and Health). 1990. National Occupational Hazard Survey, 1981-1983. Unpublished provisional data as of July 1, 1990. Department of Health, Education and Welfare, Cincinnati, OH.

Nomura, A. M. Y., L. N. Kolonel, J. H. Hankin, and C. N. Yoshizawa. 1991. Dietary Factors in Cancer of the Lower Urinary Tract. Int. J. Cancer 48: 199-205.

NTP (National Toxicology Program). 1994. Saccharin. In: Seventh Annual Report on Carcinogens, Summary 1994. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC, pp. 352-355.

Okamura, T., E. Garland, T. Masui, T. Sakata, M. St. John, and S. Cohen. 1991. Lack of Bladder Tumor Promoting Activity in Rats Fed Sodium Saccharin in AIN-76A Diet. Cancer Res. 51:1778-1782.

Olson, M., J. Johnson, and C. Reidy. 1990. Comparison of Male Rat and Human Urinary Proteins: Implications for Human Resistance to Hyaline Droplet Nephropathy. Toxicol. Appl. Pharmacol. 102:524-536.

Oser, B. 1985. Highlights in the History of Saccharin Toxicology. Food Chem. Toxicol. 23:535-542.

Otoshi, T., H. Iwata, S. Yamamoto, T. Murai, S. Yamaguchi, I. Matsui-Yuasa, S. Otani, and S. Fukushima. 1993. Severity of Promotion by Sodium Salts of Succinic Acid in Rat Urinary Bladder Carcinogenesis Correlates with Sodium Ion Concentration Under Conditions of Equal Urinary pH. Carcinogenesis 14:2277-2281.

Piper, J. M., G. M. Mantonoski, and J. Tonascia. 1986. Bladder Cancer in Young Women. Am. J. Epidemiol. 123:1033-1042.

Pitkin, R. M., D. W. Andersen, W. A. Reynolds, and L. J. Filer, Jr. 1971. Saccharin Metabolism in *Macaca mulatta.* Proc. Soc. Exp. Biol. (NY) 137:803-806.

PMC Specialties Group, Inc. 1996. High Potency Sweeteners. Information Bulletin No. FFIGEN01.

PMC Specialties Group, Inc. 1997a. Food/Feed Ingredients: SYNCAL® GS & SYNCAL® GSD (FCC, USP). Technical Bulletin No. FFI4210-11.

PMC Specialties Group, Inc. 1997b. Food/Feed Ingredients: SYNCAL® SDS (FCC, USP). Technical Bulletin No. FFI4213-14.

PMC Specialties Group, Inc. 1997c. Food/Feed Ingredients: SYNCAL® CAS (FCC, USP). Technical Bulletin No. FFI4216.

PMC Specialties Group, Inc. 1997d. Food/Feed Ingredients: SYNCAL® SDI (FCC, USP). Technical Bulletin No. FFI4215.

Prasad, O., and G. Rai. 1986. Induction of Papillary Adenocarcinoma of Thyroid in Albino Mice by Saccharin Feeding. Indian J. Exp. Biol. 24:197-199.

Renner, H. W. 1979. Possible Mutagenic Activity of Saccharin. Experientia 35:1364-1365.

Renwick, A. G. 1986. The Metabolism of Intense Sweeteners. Xenobiotica 16:1057-1071.

Renwick A. G. 1993. A Data-derived Safety (Uncertainty) Factor For the Intense Sweetener, Saccharin. Food Addit. Contam. 10:337-350.

Research Studies-USDA ERS. 1992. Sugar and Sweetener. U.S. Consumption, 1992: Synthetic Sweeteners, p. 16.

Risch, H., J. Burch, A. Miller, G. Hill, R. Steele, and G. Howe. 1988. Dietary Factors and the Incidence of Cancer of the Urinary Bladder. Am. J. Epidemiol. 127:1179-1191.

Roe, F. J. C., L. S. Levy, and R. L. Carter. 1970. Feeding Studies on Sodium Cyclamate, Saccharin and Sucrose for Carcinogenic and Tumor-Promoting Activity. Food Cosmet. Toxicol. 8:135-145.

Rossman, T. G., M. Molina, L. Meyer, P. Boone, C. B. Klein, Z. Wang, F. Li, W. C. Lin, and P. L. Kinney. 1991. Performance of 133 Compounds in the Lambda Prophage Induction Endpoint of the Microscreen Assay and a Comparison with *S. typhimurium* Mutagenicity and Rodent Carcinogenicity Assays. Mutat. Res. 260:349-367.

Roy, A. K., and O. W. Neuhaus. 1966. Identification of Rat Urinary Proteins by Zone and Immunoelectrophoresis. Proc. Soc. Exp. Biol. Med. 894-899.

Roy, A. K., and O. W. Neuhaus. 1967. Androgenic Control of a Sex-Dependent Protein in the Rat. Nature 214:618-620. (Cited by Garland et al., 1994)

Roy, A. K., T. S. Nath, N. M. Motwani, and B. Chatterjee. 1983. Age-Dependent Regulation of the Polymorphic Forms of $\alpha_{2\mu}$-Globulin. J. Biol. Chem. 258:10123-10127.

RTECS (Registry of Toxic Effects of Chemical Substances). 1996. Saccharin. Online database available from the National Library of Medicine's TOXNET system. Profile last updated on 2/7/96.

Sakai, A., and M. Sato. 1989. Improvement of Carcinogen Identification in Balb/3T3 Cell Transformation by Application of a 2-Stage Method. Mutat. Res. 214:285-296.

Salaman, M. H., and F. J. C. Roe. 1956. Further Tests For Tumour-Initiating Activity: *N,N*-Di-(2-chloroethyl)-*p*-aminophenylbutyric acid (CB1348) as an Initiator of Skin Tumour Formation in the Mouse. Br. J. Cancer. 10:363-378. (Cited by IARC, 1980)

Schmähl, D. 1973. Lack of Carcinogenic Effect of Cyclamate, Cyclohexylamine and Saccharin in Rats (German). Arzneim. Forsch. 23:1466-1470. (Cited by IARC, 1980)

Schmähl, D., and M. Habs. 1980. Absence of Carcinogenic Response to Cyclamate and Saccharin in Sprague-Dawley Rats after Transplacental Application. Arzneim. Forsch. 30:1905-1906.

Schmähl, D., and M. Habs. 1984. Investigations on the Carcinogenicity of the Artificial Sweeteners Sodium Cyclamate and Sodium Saccharin in Rats in a Two-Generation Experiment. Arzneim. Forsch. 34:604-608.

Schoenig, G. P., E. I. Goldenthal, R. G. Geil, C. H. Frith, W. R. Richter, and F. W. Carlborg. 1985. Evaluation of the Dose Response and *In Utero* Exposure to Saccharin in the Rat. Food Chem. Toxicol. 23:475-490.

Shibata, M. A., S. Tamano, Y. Kurata, A. Hagiwara, and S. Fukushima. 1989. Participation of Urinary Na^+, K^+, Ph, and l-Ascorbic Acid in the Proliferation Response of the Bladder Epithelium after the Oral Administration of Various Salts and/or Ascorbic Acid to Rats. Food Chem. Toxicol. 27:403-413.

Shioya, S., R. Nagami-Oguihara, S. Oguihara, T. Kimura, and K. Imaida. 1994. Roles of Bladder Distension, Urinary pH and Urinary Sodium Ion Concentration in Cell Proliferation of Urinary Bladder Epithelium in Rats Ingesting Sodium Salts. Food Chem. Toxicol. 32:165-171.

Sieber, S. M., and R. H. Adamson. 1978. Long-Term Studies on the Potential Carcinogenicity of Artificial Sweeteners in Non-Human Primates. In: Health and Sugar Substitutes. Guggenheim, B., Ed. Basel, Karger, pp. 266-271. (Cited by IARC, 1980)

Silverman, D. T., R. N. Hoover, and G. M. Swanson. 1983. Artificial Sweeteners and Lower Urinary Tract Cancer: Hospital vs. Population Controls. Am. J. Epidemiol. 117:326-334.

Simon, D., S. Yen, and P. Cole. 1975. Coffee Drinking and Cancer of the Lower Urinary Tract. J. Natl. Cancer Inst. 54:587-591.

Skare, J. A., and T. K. Wong. 1985. Lack of Specific Inhibition of DNA Repair in WI-38 Human Diploid Fibroblasts by Sodium Saccharin. Cancer Lett. 26:191-200.

Squire, R. A. 1985. Histopathological Evaluation of Rat Urinary Bladders from the IRDC Two-Generation Bioassay of Sodium Saccharin. Food Chem. Toxicol. 23:491.

SRI International. 1996. SRI Directory of Chemical Producers, United States. SRI International, Menlo Park, CA, pp. 311, 638.

Stoner, G. D., M. B. Shimkin, A. J. Kniazeff, J. H. Weisburger, E. K. Weisburger, and G. B. Gori. 1973. Test for Carcinogenicity of Food Additives and Chemotherapeutic Agents by the Pulmonary Tumor Response in Strain A Mice. Cancer Res. 33:3069-3085. (Cited by IARC, 1980)

Sturgeon, S. R., P. Hartge, D. T. Silverman, A. F. Kantor, W. M. Linehan, C. Lynch, and R. N. Hoover. 1994. Associations Between Bladder Cancer Risk Factors and Tumor Stage and Grade at Diagnosis. Epidemiology 5:218-225.

Suzuki, H., and N. Suzuki. 1988. Mutagenicity of Saccharin in a Human Cell Strain. Mutat. Res. 209:13-16.

Suzuki, H., and N. Suzuki. 1993. Detection of K-ras Codon 12 Mutation by Polymerase Chain Reaction and Differential Dot-Blot Hybridization in Sodium Saccharin-Treated Human RSA Cell. Biochem. Biophys. Res. Commun. 196:956-61.

Sweatman, T. W., and A. G. Renwick. 1979. Saccharin Metabolism and Tumorigenicity. Science 205:1019-1020.

Sweatman, T. W., and A. G. Renwick. 1980. The Tissue Distribution and Pharmacokinetics of Saccharin in the Rat. Toxicol. Appl. Pharmacol. 5:18-31.

Sweatman, T. W., and A. G. Renwick. 1982. Tissue Levels of Saccharin in the Rat During Two-Generation Feeding Studies. Toxicol. Appl. Pharmacol. 62:465-473.

Sweatman, T. W., A. G. Renwick, and C. D. Burgess. 1981. The Pharmacokinetics of Saccharin in Man. Xenobiotica 11:531-540.

Swenberg, J. A., D. R. Dietrich, R. M. McClain, and S. M. Cohen. 1992. Species-Specific Mechanisms of Carcinogenesis. Mechanisms of Carcinogenesis in Risk Identification. Vainio, H. et al., Eds. International Agency for Research On Cancer, Lyon, France. 477-500.

Tatematsu, M., Y. Mera, K. Kohda, Y. Kawazoe, and N. Ito. 1986. Ornithine Decarboxylase Activity and DNA Synthesis in Rats after Long Term Treatment with Butylated Hydroxyanisole, Sodium Saccharin or Phenobarbital. Cancer Lett. 33:119-124.

Taylor, J. M., and L. Friedman. 1974. Combined Chronic Feeding and Three-Generation Reproduction Study of Sodium Saccharin in the Rat (Abstract No. 200). Toxicol. Appl. Pharmacol. 29:154. Abstract.

Taylor, J. M., M. A. Weinberger, and L. Friedman. 1980. Chronic Toxicity and Carcinogenicity to the Urinary Bladder of Sodium Saccharin in the in Utero-Exposed Rat. Toxicol. Appl. Pharmacol. 54:57-75.

Thorgeirsson, U., D. Dalgard, J. Reeves, and R. Adamson. 1994. Tumor Incidence in a Chemical Carcinogenesis Study of Nonhuman Primates. Regul. Toxicol. Pharmacol. 19:130-151.

Tisdel, M. O., P. O. Nees, D. L. Harris, and P. H. Derse. 1974. Long-Term Feeding of Saccharin in Rats. In: Symposium: Sweeteners. Inglett, G. E., Ed. Avi Publishing Co., Westport, CN, pp. 145-158.

Tomasula, D. 1994. Sweet As Sugar. Chem. Mark. Rep., pp. SR22-SR23, June 27.

TRIS (Toxic Release Inventory Systems). 1996. Federal Environmental Site Liability Records. TRIS Database. 1989, 1990, 1991. "Copr. (c) West 1996, No claim to original U.S. government works."

USITC (U.S. International Trade Commission). 1991. Synthetic Organic Chemicals, United States Production and Sales, 1990. USITC Publication No. 2470. U.S. Government Printing Office, Washington, DC.

USITC (U.S. International Trade Commission). 1993. Synthetic Organic Chemicals, United States Production and Sales, 1991. USITC Publication No. 2607. U.S. Government Printing Office, Washington, DC.

USITC (U.S. International Trade Commission). 1994. Synthetic Organic Chemicals, United States Production and Sales, 1992. USITC Publication No. 2720. U.S. Government Printing Office, Washington, DC.

USITC (U.S. International Trade Commission). 1995. Synthetic Organic Chemicals, United States Production and Sales, 1994. USITC Publication No. 2933. U.S. Government Printing Office, Washington, DC.

Uwagawa, S., K. Saito, Y. Okundo, H. Kawasaki, A. Yoshitake, H. Yamada, and S. Fukushima. 1994. Lack of Induction of Epithelial Cell Proliferation by Sodium Saccharin and Sodium l-Ascorbate in the Urinary Bladder of NCI-Black-Reiter (NBR) Male Rats. Toxicol. Appl. Pharmacol. 127:182-186.

Velazquez, S. F., R. Schoeny, G. E. Rice, and J. J. Cogliano. 1996. Cancer Risk Assessment: Historical Perspectives, Current Issues, and Future Directions. Drug Chem. Toxicol. 19:161-185.

Vesely, D. L., and G. S. Levey. 1978. Saccharin Inhibits Guanylate Cyclase Activity: Possible Relationship to Carcinogenesis. Biochem. Biophys. Res. Commun. 81:1384-1389.

Viscusi, W. K. 1994. Efficacy of Labeling of Foods and Pharmaceuticals. Annu. Rev. Public Health 15:325-343.

Walker, A. M., N. A. Dreyer, E. Friedlander, J. Loughlin, K. J. Rothman, and H. I. Kohn. 1982. An Independent Analysis of the National Cancer Institute Study on Non-Nutritive Sweeteners and Bladder Cancer. Am. J. Public Health 72:376-383.

Weast, R. C., and M. J. Astle, Eds. 1980. The Merck Index, 12th ed. Merck & Co., Inc., Whitehall, NJ.

Weisburger, E. 1990. Mechanistic Considerations in Chemical Carcinogenesis. Regul. Toxicol. Pharmacol. 12:41-52.

West, R. W., W. G. Sheldom, D. W. Gaylor, M. G. Haskin, R. R. Delongchamp, and F. F. Kadlubar. 1986. The Effects of Saccharin on the Development of Neoplastic Lesions Initiated with *N*-Methyl-*N*-nitrosourea in the Rat Urothelium. Fundam. Appl. Toxicol. 7:585-600.

West, R. W., W. G. Sheldon, D. W. Gaylor, R. R. Allen, and F. F. Kadulbar. 1994. Study of Sodium Saccharin Co-Carcinogenicity in the Rat. Food Chem. Toxicol. 32:207-213.

Whysner, J., and G. M. Williams. 1996. Saccharin Mechanistic Data and Risk Assessment: Urine Composition, Enhanced Cell Proliferation, and Tumor Promotion. Pharmacol. Ther. 71:225-252.

Williams, G. M., and J. Whysner. 1996. Epigenetic Carcinogens: Evaluation and Risk Assessment. Exp. Toxicol. Pathol. 48:189-195.

Wright, S. C., J. Zhong, and J. W. Larrick. 1994. Inhibition of Apoptosis as a Mechanism of Tumor Promotion. FASEB J. 8:654-60.

Wynder, E. L., and R. Goldsmith. 1977. The Epidemiology of Bladder Cancer. A Second Look. Cancer 40:1246-1268.

Wynder, E. L., and S. D. Stellman. 1980. Artificial Sweetener Use and Bladder Cancer: A Case-Control Study. Science 207:1214-1216.

Yanagisawa, K., K. Nishio, and S. Gotoh. 1987. Screening for Carcinogens by the DNA Synthesis Inhibition Test Using Human Fibroblasts. Mutat Res. 183:89-94.

Yu, A., T. Hashimura, Y. Nishio, H. Kanamaru, S. Fukuzawa, and O. Yoshida. 1992. Anti-Promoting Effect of Nordihydroguaiaretic acid on *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine and Sodium Saccharin-Induced Rat Urinary Bladder Carcinogenesis. Jpn. J. Cancer Res. 83:944-948.

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

(SACCHARIN, SODIUM SACCHARIN, CALCIUM SACCHARIN & *ortho*-TOLUENESULPHONAMIDE)

1. Chemical and Physical Data

Saccharin[1]

1.1 Synonyms and trade names

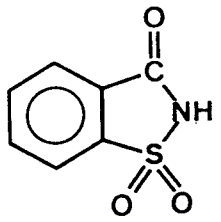
Chem. Abstr. Services Reg. No.: 81-07-2

Chem. Abstr. Name: 1,2-Benzisothiazol-3(2*H*)-one,1,1-dioxide

Synonyms: Anhydro-*ortho*-sulphaminebenzoic acid; 1,2-benzisothiazolinone, 1,1-dioxide; 1,2-benzisothiazolin-3-one, 1,1-dioxide; 3-benzisothiazolinone 1,1-dioxide; benzoic sulphimide; *ortho*-benzoic sulphimide; benzoic sulphinide; benzosulphimide; benzo-2-sulphimide; *ortho*-benzosulphimide; benzo-sulphinide; *ortho*-benzoyl sulphimide; 1,2-dihydro-2-ketobenzisosulphonazole; 2,3-dihydro-3-oxobenzisosulphonazole; 3-hydroxybenzisothiazole-*S,S*-dioxide; insoluble saccharin; saccharimide; saccharin acid; saccharine; saccharin insoluble; *ortho*-sulphobenzimide; *ortho*-sulphobenzoic acid imide; 2-sulphobenzoic imide

Trade names: Assugrin vollsuss (also contains sodium cyclamate); Garantose; Glucid; Gluside; Hermesetas; Kandiset; Natreen (also contains sodium cyclamate); Sacarina; 550 Saccharin; Saccharina; Saccharinol; Saccharinose; Saccharol; Saxin; Sucre Edulcor; Sucrette; Sykose; Zaharina

1.2 Structural and molecular formulae and molecular weight



$C_7H_5NO_3S$ Mol. wt: 183.2

[1] The name 'saccharin' is sometimes (e.g., in government regulations) applied to the ammonium, calcium and sodium salts as well as to the free acid.

1.3 Chemical and physical properties of the pure substance

From Wade (1977) and Windholz (1976), unless otherwise specified

(a) Description: White crystalline powder with an intensely sweet taste

(b) Melting-point: 228.8-229.7°C

(c) Spectroscopy data: Broad peak at 267.3 nm (E_1^1 85.7)

(d) Solubility: Soluble in water (1g in 290 ml), boiling water (1 in 25), acetone (1 in 12), ethanol (1 in 30) and glycerol (1 in 50); slightly soluble in chloroform and in diethyl ether; soluble in dilute aqueous solutions of ammonia and alkaline hydroxides and carbonates

(e) pH of aqueous solution: Acid to litmus (National Research Council, 1972)

(f) Sweetness: Dilute aqueous solution is about 500 times sweeter than a solution containing an equal concentration by weight of sucrose.

1.4 Technical products and impurities

Saccharin is available in the US as saccharin insoluble powder FCC (Food Chemicals Codex), which meets or exceeds the following specifications: 98-101% active ingredient on an anhydrous basis, a maximum of 100 mg/kg toluenesulphonamides, 30 mg/kg selenium, 10 mg/kg heavy metals (as lead), and 3 mg/kg arsenic. It passes a colour-precipitate test for benzoic and salicylic acids and a colour test for readily carbonizable substances (National Research Council, 1972, 1974; The Sherwin-Williams Co., 1978a).

Various national and international pharmacopoeias give specifications for the purity of saccharin in pharmaceutical products. For example, saccharin is available in the US as a USP grade containing 98-101% active ingredient on an anhydrous basis (US Pharmacopeial Convention, Inc., 1975).

In France, saccharin is available as non-nutritive sweetening tablets containing: (1) 20 mg saccharin, 8 μg disodium methylarsonate (added to reduce its bitter taste), and 0.2 mg lithium chloride; and (2) 25 mg saccharin, 75 mg sodium bicarbonate and 0.15 mg sodium arsenate. No limits have been set on the content of *ortho*-toluenesulphonamide or other impurities in saccharin.

In the Federal Republic of Germany, saccharin meets the following specifications: 98% active ingredient on an anhydrous basis and a maximum of 10 mg/kg each of *ortho*- and *para*-toluenesulphonamides and 30 mg/kg selenium (Bundesminister der Justiz, 1979).

In the UK, no maximum limit for the content of *ortho*-toluenesulphonamide has been regulated at present; however, all existing regulations for non-nutritive sweeteners are under review.

Impurities have been identified in commercial saccharin made by both the Remsen-Fahlberg process (known to be used in Japan, the Republic of Korea and the UK) and the Maumee process (known to be used in the US) (US International Trade Commission, 1977a). Table 1 is a summary of published data on impurities in saccharin and sodium saccharin.

Sodium saccharin

1.1 Synonyms and trade names

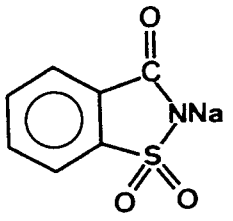
Chem. Abstr. Services Reg. No.: 128-44-9

Chem. Abstr. Name: 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, sodium salt

Synonyms: 1,2-Benzisothiazolin-3-one,1,1-dioxide, sodium salt; saccharin sodium; saccharin soluble; sodium benzosulphimide; sodium 2-benzosulphimide; sodium *ortho*-benzosulphimide; sodium saccharide; sodium saccharinate; sodium saccharine; soluble saccharin; 2-sulphobenzoic imide, sodium salt

Trade names: Cristallose; Crystallose; Dagutan; Kristallose; ODA; Saccharinnatrium; Saccharoidum Natricum; Saxin; Soluble Gluside; Succaril (also contains sodium cyclamate); Sucra; Sweeta; Sykose; Willosetten

1.2 Structural and molecular formulae and molecular weight



$C_7H_4NNaO_3S$ Mol. wt: 205.2

Table 1. Reported impurities in saccharin and sodium saccharin

Impurity	Approx. concentration reported (mg/kg)	Synthetic method[a]	Reference
ortho-Toluenesulphonamide	up to 6000 (before 1973-74)	RF	National Research Council/National Academy of Sciences (1978)
	⩽25 (more recently)	RF	National Research Council/National Academy of Sciences (1978)
			Stavrić *et al.* (1976)
	⟨0.1	M	Stavrić *et al.* (1976)
para-Toluenesulphonamide	⩽5	RF	National Research Council/National Academy of Sciences (1978)
	⟨0.2	M	Riggin *et al.* (1978)
	I[b]	RF	Stavrić *et al.* (1976)
1,2-Benzisothiazol-1,1-dioxide	⩽10	RF	National Research Council/National Academy of Sciences (1978)
	I	RF	Stavrić *et al.* (1976)
			Riggin *et al.* (1978)
1,2-Benzisothiazoline-1,1-dioxide	1 - 10	RF	National Research Council/National Academy of Sciences (1978)
			Riggin *et al.* (1978)
	I	RF	Stavrić *et al.* (1976)
	I	M	Riggin *et al.* (1978)
3-Aminobenzisothiazol-1,1-dioxide	2 - 19	RF	Riggin *et al.* (1978)
	1	RF	National Research Council/National Academy of Sciences (1978)
	4 - 17	M	Riggin *et al.* (1978)

Table 1 (contd)

Impurity	Approx. concentration reported (mg/kg)	Synthetic method[a]	Reference
5-Chlorosaccharin	I	RF	National Research Council/National Academy of Sciences (1978)
			Riggin *et al.* (1978)
	< 25	M	Riggin *et al.* (1978)
6-Chlorosaccharin	I	M, RF	National Research Council/National Academy of Sciences (1978)
			Riggin *et al.* (1978)
Ammonium saccharin	50 - 500	M	Riggin *et al.* (1978)
Methyl saccharin	0.16	M	Riggin *et al.* (1978)
Diphenyl sulphone	1 - 7	RF	Riggin *et al.* (1978)
ortho,ortho'-Ditolylsulphone *ortho,meta'*- Ditolylsulphone *ortho,para'*- Ditolylsulphone	< 50 (total)	RF	Stavrić *et al.* (1976) Riggin *et al.* (1978)
meta,para'-Ditolylsulphone *para,para'*-Ditolylsulphone	< 5 (total)	RF	National Research Council/National Academy of Sciences (1978)
ortho-Sulphamoylbenzoic acid	0 - 181	RF	Riggin *et al.* (1978)
	21 - 41	M	Riggin *et al.* (1978)
para-Sulphamoylbenzoic acid	10 - 1057	RF	Riggin *et al.* (1978)
	I	M	Riggin *et al.* (1978)

Table 1 (contd)

Impurity	Approx. concentration reported (mg/kg)	Synthetic method[a]	Reference
ortho-Chlorobenzoic acid	I	M, RF	National Research Council/National Academy of Sciences (1978)
			Riggin *et al.* (1978)
ortho-Sulphobenzoic acid	I	M, RF	Riggin *et al.* (1978)
ortho-Sulphobenzoic acid, ammonium salt	I	M, RF	National Research Council/National Academy of Sciences (1978)
n-Tetracosane	I	RF	Stavrić *et al.* (1976)
			Riggin *et al.* (1978)
Bis(4-carboxyphenyl)sulphone	10	RF	Riggin *et al.* (1978)
Toluene-2,4-disulphonamide	I	RF	National Research Council/National Academy of Sciences (1978)
Saccharin-*ortho*-toluenesulphonamide	I	RF	National Research Council/National Academy of Sciences (1978)
Saccharin-6-sulphonamide	I	RF	National Research Council/National Academy of Sciences (1978)
N-Methyl-*ortho*-toluenesulphonamide	I	RF	National Research Council/National Academy of Sciences (1978)
Methyl-*ortho*-chlorobenzoate	I	RF	National Research Council/National Academy of Sciences (1978)
4,4'-Dibenzoylsulphone	I	RF	National Research Council/National Academy of Sciences (1978)
2- or 3- Carboxy thiaxanthone-5-dioxide	I	RF	National Research Council/National Academy of Sciences (1978)

Table 1 (contd)

Impurity	Approx. concentration reported (mg/kg)	Synthetic method[a]	Reference
ortho-Sulphobenzamide	I	RF	National Research Council/National Academy of Sciences (1978)
Methyl-*ortho*-sulphamoylbenzoate	I	RF	National Research Council/National Academy of Sciences (1978)
Methyl-*N*-methylsulphamoyl-benzoate	I	RF	National Research Council/National Academy of Sciences (1978)
Saccharin-*ortho*-toluene-sulphoxylimide	I	RF	Riggin *et al.* (1978)
Various phthalate esters	0.04	M	Riggin *et al.* (1978)
Trioctyl phosphate	0.06	M	Riggin *et al.* (1978)
Various fatty acid amides	0.75	M	Riggin *et al.* (1978)
Mineral oil (saturated hydro-carbons)	5.0	M	Riggin *et al.* (1978)
Butylated hydroxytoluene	0.04	M	Riggin *et al.* (1978)
Butylated hydroxyanisole	0.01	M	Riggin *et al.* (1978)
Methyl anthranilate	0.05	M	Riggin *et al.* (1978)
ortho-Chlorobenzamide	0.02	M	Riggin *et al.* (1978)
Trichlorobenzene	0.05	M	Riggin *et al.* (1978)
2,6-Di-*tert*-butyl-*para*-benzo-quinone	0.005	M	Riggin *et al.* (1978)

Table 1 (contd)

Impurity	Approx. concentration reported (mg/kg)	Synthetic method[a]	Reference
Lead Selenium Silver Arsenic Bismuth Cadmium Copper Mercury Tin	Below Food Chemicals Codex specifications (< 0.5)	M	Riggin *et al.* (1978)

[a] RF - Remsen-Fahlberg method; M - Maumee method

[b] I - Identified but not quantified

1.3 Chemical and physical properties of the dihydrate

From Wade (1977), unless otherwise specified

(a) *Description:* White crystalline powder with an intensely sweet taste

(b) *Melting-point:* Greater than 300°C (decomposes) (Beck, 1969)

(c) *Solubility:* Soluble in water (1g in 1.5 ml) and ethanol (1 in 50)

(d) *pH of aqueous solution:* Neutral or alkaline to litmus but not alkaline to phenolphthalein (Windholz, 1976)

(e) *Sweetness:* Dilute aqueous solution is about 300 times sweeter than a solution containing an equal concentration by weight of sucrose.

1.4 Technical products and impurities

Sodium saccharin FCC (Food Chemicals Codex) is available in the US in four grades: spray-dried, containing 3.0% moisture; powder, containing 5.0-5.8% moisture; pelletized, containing 10.5-11.5% moisture; and granular, containing 14.0-15.0% moisture. Each of these grades meets or exceeds the following Food Chemicals Codex specifications: 98-101% active ingredient on an anhydrous basis, 3-15% water, 100 mg/kg toluenesulphonamides, 30 mg/kg selenium, 10 mg/kg heavy metals (as lead) and 3 mg/kg arsenic. They pass a colour-precipitate test for benzoates and salicylates, a colour test for readily carbonizable substances and a colour test for alkalinity (National Research Council, 1972, 1974; The Sherwin-Williams Co., 1977a, 1978b). An industrial grade is also marketed; however, no specifications were available to the Working Group (The Sherwin-Williams Co., 1977b).

Various national and international pharmacopoeias give specifications for the purity of sodium saccharin in pharmaceutical products. For example, it is available in the US as a National Formulary (NF) grade containing 98-101% active ingredient on an anhydrous basis. Tablets are available in 15, 30 and 60 mg doses which contain 95-110% of the stated amount of sodium saccharin (National Formulary Board, 1975).

Until 1970, when the use of cyclamates in food was banned in the US, sodium saccharin was also available in: (1) aqueous solutions containing about 0.6% sodium saccharin combined with about 6% sodium cyclamate and (2) tablets containing about 5 mg sodium saccharin combined with about 50 mg sodium cyclamate (National Formulary Board, 1970).

Sodium saccharin available in Europe has the following specifications: purity, 98-101% on a dried basis; loss on drying, 15% max; a minimum of 32% sulphate ash on a dried basis; a maximum of 200 mg/kg sulphate, 200 mg/kg chloride, 30 mg/kg selenium, 10 mg/kg heavy metals, 10 mg/kg *ortho*-toluenesulphonamide and 2 mg/kg arsenic; and no visible contamination by a thin-layer chromatographic test.

In France, sodium saccharin is available as a non-nutritive sweetening tablet containing 13 mg sodium saccharin and 20 μg 5-methoxyresorcinol; and a tablet containing 50 mg sodium cyclamate and 5 mg sodium saccharin.

It is available in the UK as an aqueous solution and in 12.5 mg tablets (Wade, 1977).

In the Federal Republic of Germany, sodium saccharin meets the following specifications: 98% active on an anhydrous basis and a maximum of 10 mg/kg *ortho*-toluenesulphonamide and 30 mg/kg selenium (Bundesminister der Justiz, 1979).

In the UK, no maximum limit for the content of *ortho*-toluenesulphonamide in sodium saccharin has been regulated at present; however, all existing regulations for non-nutritive sweeteners are under review.

Sodium saccharin available in Japan must be 99% pure and contain a maximum of 100 mg/kg *ortho*-toluenesulphonamide; the *ortho*-toluenesulphonamide content at present has been found to be in the range of 15-20 mg/kg.

Calcium saccharin

1.1 Synonyms and trade names

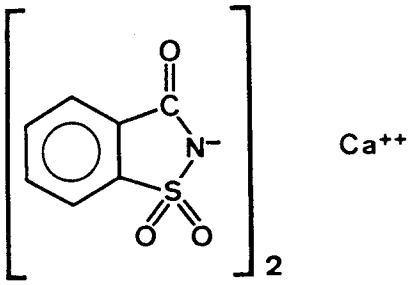
Chem. Abstr. Services Reg. No.: 6485-34-3

Chem. Abstr. Name: 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, calcium salt

Synonyms: 1,2-Benzisothiazolin-3-one,1,1-dioxide, calcium salt; calcium benzosulphimide; calcium 2-benzosulphimide; calcium-*ortho*-benzosulphimide; calcium saccharina; calcium saccharinate; calcium saccharine; saccharin calcium; 2-sulphobenzoic imide, calcium salt

Trade name: Daramin

1.2 Structural and molecular formulae and molecular weight



$C_{14}H_8N_2CaO_6S_2$ Mol. wt: 404.4

1.3 Chemical and physical properties of the hydrate

From Beck (1969) and Wade (1977)

(a) Description: White crystalline powder with intensely sweet taste

(b) Solubility: Soluble in water (1 g in 1.5 ml) and 92% ethanol (1 in 33)

(c) Sweetness: Dilute aqueous solution is about 300 times sweeter than a solution containing an equal concentration by weight of sucrose.

1.4 Technical products and impurities

In 1975, calcium saccharin that met specifications for the US National Formulary (NF) grade was required to contain 98-101% active ingredient on an anhydrous basis, 3-15% water, a maximum of 3 mg/kg arsenic, 30 mg/kg selenium and 10 mg/kg heavy metals, and to pass a colour-precipitate test for benzoates and salicylates and a colour test for readily carbonizable substances (National Formulary Board, 1975).

Until 1970, when the use of cyclamates in food was banned in the US, calcium saccharin was also available in: (1) aqueous solutions containing about 0.6% calcium saccharin combined with about 6% calcium cyclamates, and (2) tablets containing about 5 mg calcium saccharin combined with about 50 mg calcium cyclamate (National Formulary Board, 1970).

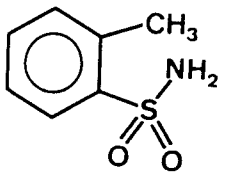
ortho-Toluenesulphonamide

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 88-19-7

Chem. Abstr. Name: 2-Methylbenzenesulfonamide

1.2 Structural and molecular formulae and molecular weight



$C_7H_9NO_2S$ Mol. wt: 171.2

1.3 Chemical and physical properties of the pure substance

From Hawley (1977) and Weast (1977), unless otherwise specified

(a) Description: Colourless crystals

(b) Melting-point: 156.3°C

(c) Spectroscopy data: Ultra-violet spectrum has two sharp peaks at 268 and 275 nm (in methanol) (Grasselli, 1973)

(d) Solubility: Soluble in ethanol; slightly soluble in water and diethyl ether

1.4 Technical products and impurities

ortho-Toluenesulphonamide is not produced commercially as a separate chemical in the US; however, a product consisting of a mixture of unknown proportions of the *ortho*- and *para*-isomers of toluenesulphonamide is produced in the US as fine, white-to-light cream granular particles containing 1.0% maximum moisture, with the following properties: flash-point, 215°C; melting-point, 105°C; boiling-point (10 mm), 214°C; and pH, 4.0 min (Monsanto Industrial Chemicals Co., undated).

ortho-Toluenesulphonamide available in Japan has the following specifications: melting-point, 155°C min; water, 0.5% max; ash, 0.2% max; *para*-toluenesulphonamide, 2% max.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

SACCHARIN, SODIUM SACCHARIN AND CALCIUM SACCHARIN

(a) Production

Saccharin was first synthesized in 1879 by Remsen & Fahlberg by: (1) reaction of toluene with chlorosulphonic acid to produce *ortho-* and *para*-toluenesulphonyl chlorides; (2) separation of the *ortho*-isomer followed by treatment with ammonia to form *ortho*-toluenesulphonamide; (3) oxidation to *ortho*-sulphamoylbenzoic acid, which, on heating, was cyclized to saccharin (Remsen & Fahlberg, 1879). Essentially the same method was reportedly used for commercial production by one US company until 1972 and is still used by the six producing companies in Japan, the three producing companies in the Republic of Korea (US International Trade Commission, 1977a) and the sole producer in the UK.

Currently, saccharin and sodium saccharin are produced commercially in the US only by the Maumee process. In this process, methyl anthranilate (made either by the methylation of anthranilic acid, the reaction of phthalic anhydride with ammonia, sodium hypochlorite and methanol, or the reaction of isatoic anhydride with methanol) is diazotized by treatment with sodium nitrite and hydrochloric acid to form 2-carbomethoxybenzenediazonium chloride. Sulphonation of this produces 2-carbomethyoxybenzenesulphinic acid, which is converted to 2-carbomethoxybenzenesulphonyl chloride with chlorine. Amidation of this sulphonylchloride, followed by acidification, forms saccharin, which is treated with either sodium hydroxide or sodium bicarbonate to produce sodium saccharin (National Research Council/National Academy of Sciences, 1978). Calcium saccharin can be produced by the reaction of calcium hydroxide with saccharin.

Saccharin and saccharin sodium have been produced commercially in the US for over 80 years (Crammer & Ikan, 1977); calcium saccharin was first produced commercially in the US in 1953 (US Tariff Commission, 1954). From an estimated level of 180 thousand kg in 1957, US production of saccharin (all forms) increased gradually to an estimated 2040 thousand kg in 1970. Only one US company reported commercial production of an undisclosed amount (see preamble, p. 20) of saccharin and sodium saccharin in 1977 (US International Trade Commission, 1978a); one source has estimated that a total of 2177 thousand kg were produced in that year (National Research Council/National Academy of Sciences, 1978). Commercial production of calcium saccharin was last reported by one company in the US in 1974 (US International Trade Commission, 1976a).

US imports of saccharin (all forms) increased from 45 thousand kg in 1955 to 500 thousand kg in 1963; after decreasing to a low of 310 thousand kg in 1967, imports increased to a high of 1540 thousand kg in 1974. By 1977, US imports of saccharin (all forms), chiefly from Japan (68%) and the Republic of Korea (19%), amounted to 1380 thousand kg (US Department of Commerce, 1978). A total of 214 thousand kg saccharin, 716 thousand kg sodium saccharin and 61 thousand kg calcium saccharin were imported through principal US customs districts in 1977 (US International Trade Commission, 1978b).

Saccharin and sodium saccharin are not produced commercially in Canada; however, they are imported (primarily from the US and Japan).

Annual production of saccharin and sodium saccharin in Europe is estimated to be in the range of 100-1000 thousand kg for each chemical; the Federal Republic of Germany, Spain and the UK are believed to be the major producing countries. Annual saccharin production in the Federal Republic of Germany was 30 thousand kg in 1894, increased to 300 thousand kg in 1922, decreased to 96 thousand kg in 1934, rose to 500 thousand kg in 1944 and dropped again to 27 thousand kg in 1965 (Crampton, 1975). In the UK, commercial production of saccharin and sodium saccharin was first reported in 1916.

Saccharin and sodium saccharin have been produced commercially in Japan since before 1945. In 1978, six Japanese manufacturers produced an estimated 2840 thousand kg each of saccharin and sodium saccharin, and about 1930 thousand kg of sodium saccharin were exported.

Saccharin and sodium saccharin are also produced commercially in Taiwan; however, no information was available on the quantities produced. In 1976, saccharin was produced commercially by three companies in the Republic of Korea (US International Trade Commission, 1977a).

(b) Use

Saccharin was initially used as a non-nutritive sweetening agent in 1907, but prior to that it had been used as an antiseptic and preservative to retard fermentation in food (National Research Council/National Academy of Sciences, 1978). Since 1970, when the use of cyclamates in food was banned in the US, the food grades of the various forms of saccharin have been used as non-nutritive sweetening agents in a variety of applications (National Formulary Board, 1970, 1975; National Research Council/National Academy of Sciences, 1978).

The US consumption pattern for saccharin (all forms) in 1976 has been estimated as follows: 77% in food uses: 45% in soft drinks, 18% in 'tabletop' sweeteners, and 14% in other foods such as fruits, premixes, juices, sweets, chewing gum and jellies; and 23% in nonfood items: 10% in cosmetics such as toothpaste, mouthwash and lipstick, 7% in pharmaceuticals such as coatings on pills, 2% in smokeless tobacco products such as chewing tobacco and snuff, 2% in electroplating, 1% in cattle feed and 1% in miscellaneous uses (National Research Council/National Academy of Sciences, 1978).

Typical concentrations of sodium saccharin in food products are as follows: sugar substitutes, 13.5 mg/teaspoon sugar sweetening equivalent; carbonated soft drinks, 9.5 mg/fluid ounce; still soft drinks, 5.5 mg/fluid ounce; jams and jellies, 4.5 mg/teaspoon; chewing gum, 2.2 mg/stick.

It has been reported that saccharin itself (as opposed to its salts) has been used in the sweetening of pharmaceutical tablets and in the processing of tobacco (Anon., 1963).

Industrial grade sodium saccharin is reportedly used as a brightener in nickel-plating baths, as an antistatic agent in plastics and textiles, as a polymer modifier and accelerator in photosensitive dispersions, and as a light fastness aid in nylon dyes (The Sherwin-Williams Co., 1977b)

It has been reported that before 1977 approximately 205 thousand kg of saccharin were consumed in Canada annually for food and industrial uses (Canadian Health & Welfare Department, 1977).

Consumption of saccharin and sodium saccharin in western Europe is estimated to be in the range of 100-1000 and 1000-5000 thousand kg, respectively.

In Japan, saccharin is also used as a chemical intermediate for the fungicide probenazole, which is used commercially in controlling rice blast (Yamada, 1975). Of the estimated 914 thousand kg of saccharin sodium used in Japan in 1978, approximately 60% was used in foods and beverages and 40% in miscellaneous uses (e.g., industrial applications and pharmaceutical uses).

In the US, saccharin (including the calcium, sodium and ammonium salts) was approved for use in foods under the 1958 Food Additives Amendment to the Food, Drug, and Cosmetic Act. Under the provisions of this amendment, saccharin was included in those substances that had been in use prior to 1958 and that had been accorded GRAS (generally recognized as safe) status (National Research Council/National Academy of Sciences, 1978). On 1 February, 1972, questions concerning the safety of saccharin prompted the US Food and Drug Administration (FDA) to remove saccharin from GRAS status and to establish the following interim food additive regulation. The use of saccharin and its sodium, calcium

and ammonium salts as sweetening agents in food in the US is permitted, provided the amounts do not exceed the following: 12 mg per oz in beverages and in bases or mixes when prepared for consumption in accordance with directions; 20 mg of additive (calculated as saccharin) for each expressed teaspoonful of sugar sweetening equivalency, as a sugar substitute for cooking or table use; and 30 mg per serving in processed foods. The additives are intended for use in vitamin tablets, chewing gum and in nonstandardized bakery products and must provide labelling, including the name of the additive, concentration (expressed as saccharin) and adequate directions for use (US Food & Drug Administration, 1977a, 1978).

On 7 January 1977, an amendment to the interim food additive regulation for saccharin and its salts was proposed to establish a tolerance for 25 mg *ortho*-toluenesulphonamide per kg saccharin (US Food & Drug Administration, 1977b).

In compliance with the Delaney clause of the amendment, which prohibits the use in food of any ingredient shown to cause cancer in animals or man, the FDA published a proposal to ban the food use of saccharin on 15 April1977 (US Food & Drug Administration, 1977c). Final regulations by the FDA are now pending additional study as a result of the Saccharin Study and Labeling Act, passed by the US Congress in November 1977. The act requires studies of the impurities and toxicity of saccharin, and of the health benefits, if any, resulting from the use of non-nutritive sweeteners. It also requires certain labels and notices for foods containing saccharin (effective 21 February 1978) and prohibits action restricting the continued use of saccharin as a component of food, drugs and cosmetics for 18 months (Anon., 1978; US Food & Drug Administration, 1978; US International Trade Commission, 1977a).

In 1977, the Joint FAO/WHO Expert Committee on Food Additives re-evaluated the previously established unconditional acceptable daily intake (ADI) of 0-5 mg/kg bw and the conditional ADI of 0-15 mg/kg bw for saccharin and established a temporary acceptable daily saccharin intake of 0-2.5 mg/kg bw until further testing is completed (WHO, 1978).

On 29 March 1978, the Commission of the European Communities recommended to its member states the temporary ADI of 0-2.5 mg/kg bw proposed by the Joint FAO/WHO Expert Committee on Food Additives (Commission of the European Communities, 1978).

The regulatory status of non-nutritive sweeteners containing saccharin and/or cyclamates in various countries is outlined in the Appendix to the **General Remarks on the Substances Considered**, p. 39.

ORTHO-TOLUENESULPHONAMIDE

(a) Production

ortho-Toluenesulphonamide was prepared in 1879 by Remsen and Fahlberg by: (1) reaction of toluene with chlorosulphonic acid to produce *ortho*- and *para*-toluenesulphonyl chlorides, and (2) separation of the *ortho*-isomer followed by treatment with ammonia to form *ortho*-toluenesulphonamide (Remsen & Fahlberg, 1879). Essentially the same procedure is believed to have been used for its commercial production in the US and is still used for its commercial production in Japan.

In the US, *ortho*-toluenesulphonamide was produced in commercial quantities from 1921 (US Tariff Commission, 1922) until 1975 (US International Trade Commission, 1977b); and an *ortho,para*-toluenesulphonamide mixture has been produced commercially since 1939 (US Tariff Commission, 1940). Only one US company reported commercial production of an undisclosed amount (see preamble, p. 20) of *ortho*-toluenesulphonamide in 1975 (US International Trade Commission, 1977b) and of *ortho-, para*-toluenesulphonamide mixtures in 1977 (US International Trade Commission, 1978a).

In 1973, US imports of *ortho,para*-toluenesulphonamides through the principal US customs districts were reported as 70.2 thousand kg '*ortho,para*-toluenesulphonamide' and 77.1 thousand kg '*ortho,para*-toluenesulphonamide mixtures (Topcizer no.2)' (US Tariff Commission, 1974). In 1974, imports of the latter were reported to have been 18.6 thousand kg (US International Trade Commission, 1976b). No imports have been reported in recent years.

ortho-Toluenesulphonamide is believed to be produced commercially in Italy and The Netherlands; however, no information was available on the quantities produced.

ortho-Toluenesulphonamide has been prepared commercially in Japan since before 1945. In 1978, three Japanese manufacturers produced an estimated 3 million kg *ortho*-toluenesulphonamide and about 2 million kg of the *ortho,para*-toluenesulphonamide mixture.

(b) Use

Until 1972, *ortho*-toluenesulphonamide was used in the US as a chemical intermediate for the commercial production of saccharin (US International Trade Commission, 1977a). The *ortho*- and *para*-toluenesulphonamide mixture is used as a reactive plasticizer in hot-melt adhesives to improve the flow properties of thermosetting resins (e.g., melamine, urea and phenolic resins) and to impart flexibility to coatings based on resins made from casein, shellac, zein and soya protein (Monsanto Industrial Chemicals Co., undated). This mixture is also believed to be used as a carrier in fluorescent pigments.

ortho-Toluenesulphonamide is used as a starting material for the commercial production of saccharin in Japan, the Republic of Korea (US International Trade Commission, 1977a) and the UK. In Japan, 60% of the *ortho*-toluenesulphonamide consumed is used as a chemical intermediate for the commercial production of saccharin, and 40% (in the form of the *ortho*- and *para*-toluenesulphonamide mixture) is used as a plasticizer and pigment carrier.

The US Food and Drug Administration has classified the mixture of *ortho*- and *para*-toluenesulphonamides as a safe component of adhesives used in articles intended for packaging, transporting or holding food if used in quantities not exceeding the limits of good manufacturing practice (US Food & Drug Administration, 1978).

(c) Occurrence

Saccharin, sodium and calcium saccharin and *ortho*-toluenesulphonamide do not occur as natural products.

2.3 Analysis

Typical methods for the analysis of saccharin, sodium saccharin and calcium saccharin are summarized in Table 2; methods for *ortho*-toluenesulphonamide are listed in Table 3.

Table 2. Methods for the analysis of saccharin, sodium saccharin and calcium saccharin

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Bulk chemical	Dissolve saccharin (hot water), add phenolphthalein	Titration (sodium hydroxide)	-	WHO, 1976
Bulk chemical	Dry saccharin salts, dissolve (acetic acid), add crystal violet-glacial acetic acid	Titration (perchloric acid)	-	WHO, 1976
Pharmaceutical preparations	Add hydrochloric acid, perform series of extractions (isopropyl ether), filter through anhydrous sodium sulphate, evaporate, dissolve (methanol), evaporate, add *N,O*-bis-(trimethylsilyl)acetamide, add internal standard (*n*-octacosane)	GC/FID	-	Ratchik & Viswanathan, 1975
Multivitamin tablet	Powder, extract (diethyl ether), add hydrochloric acid, extract (diethyl ether), filter through anhydrous sodium sulphate, evaporate, dissolve (ethanol)	PGC/FID/TCD	3.0 μg	Szinai & Roy, 1976
Liquid sweetener concentrates	Inject directly	HPLC/UV (254 nm)	0.014 μg	Smyly *et al.*, 1976
Sweetener tablets	Powder, dissolve (sodium carbonate solution)	UV (325-220 nm)	-	Hussein *et al.*, 1976
Toothpaste	Dilute (water), centrifuge	HPLC/UV (254 nm)	-	Simko, 1977
Soft drinks	Expel gases, acidify (sulphuric acid), perform series of extractions (diethyl ether) and washings (water), evaporate, add ethanol, copper (II) acetate solution and phenothiazine solution, heat, add ethanol and xylene, dilute (water), agitate, dry xylene layer with anhydrous sodium sulphate	VIS (510 nm)	-	Tanaka *et al.*, 1977

Table 2 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Soft drinks	Expel gases	HPLC/UV (254 nm)	0.014 μg	Smyly *et al.*, 1976
Soft drinks	Extract (ethyl acetate), basify (sodium hydroxide)	MECA (384 nm)	2 mg/l	Belcher *et al.*, 1976
Soft drinks	Expel gases	PGC/FID/TCD	1.0 μg	Szinai & Roy, 1976
Beverages	Acidify (hydrochloric acid), perform series of extractions (chloroform) and washings (water), evaporate, dissolve (sodium carbonate solution)	UV (350-220 nm)	-	Hussein *et al.*, 1976
Sweetened wine	Evaporate, add sulphuric acid, extract (diethyl ether), evaporate, add internal standard (benzenesulphonic acid)	HPLC/UV (254 nm)	25 mg/l	Tenenbaum & Martin, 1977
Chewing gum	Add internal standard (dilute aminobenzoic acid) and toluene, agitate until disintegration, separate aqueous phase, filter	HPLC/UV (254 nm)	-	Eng *et al.*, 1977
Chewing gum	Freeze, pulverize, dilute (water), acidify (hydrochloric acid), perform series of extractions (chloroform) and washings (water), evaporate, dissolve (sodium carbonate solution)	UV (325-220 nm)	-	Hussein *et al.*, 1976
Urine	Add tetrabutylammonium hydrogen sulphate (buffer, pH 7.4), agitate with methyl iodide and dichloromethane, dilute with ethyl acetate, evaporate, add ethyl acetate and saturated silver sulphate solution, agitate	GC/ECD	10 μg/l	Hartvig *et al.*, 1978

Abbreviations: GC/FID - gas chromatography/flame-ionization detection; HPLC/UV - high-pressure liquid chromatography/ultra-violet spectrometry; VIS - visible spectrometry; MECA - molecular emission cavity analysis spectrometry; GC/EC - gas chromatography/electron capture detection; PGC/FID/TCD - pyrolysis gas chromatography/flame-ionization detection/thermal conductivity detection; UV - ultra-violet spectrometry

Table 3. Methods for the analysis of *ortho*-toluenesulphonamide

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Saccharin or sodium saccharin	Neutralize if acid form (sodium hydroxide), dissolve (water), extract (dichloromethane), add 5% sodium bicarbonate solution, agitate, separate organic phase , evaporate, add methylene chloride	GC/FID	0.05 mg/kg	Janiak *et al.*, 1978
	Neutralize if acid form (15% sodium hydroxide), perform series of extractions (ethyl acetate), evaporate, add internal standard (caffeine, ethyl acetate solution)	GC/FID	0.05 mg/kg	Stavrić *et al.*, 1974
	Neutralize if acid form (sodium hydroxide), add 0.5% disodium hydrogen phosphate, perform series of extractions (dichloromethane), evaporate, add ethanol, concentrate, react residue with TRI-SIL reagent	UV	0.01 mg/ml	Jacin, 1975
Nickel-plating electrolyte	Extract (chloroform), evaporate, dissolve (water), separate by TLC (benzene:ethyl acetate:ethanol, 50:20:1, and diethyl ether:ethyl acetate, 1:1)	EP/TLC (269 nm)	–	Mockute & Bernotiene, 1976

Abbreviations: GC/FID - gas chromatography/flame-ionization detection; UV - ultra-violet spectrometry; EP/TLC - extraction photometry/thin-layer chromatography

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

SACCHARIN AND SODIUM SACCHARIN

(a) Oral administration

Single-generation exposure

Mouse: Groups of 50 female Swiss mice received 0 or 5% saccharin made by the Remsen-Fahlberg method (British Saccharin Sales Co. Ltd, UK) in the diet for 18 months, at which time the survivors were killed. Average survival rates were not affected, and tumour incidences were similar in tested and control animals. No pathological alterations were observed macroscopically in the urinary bladder (Roe *et al.*, 1970) [The Working Group noted that the urinary bladders were not examined histologically].

As part of a multigeneration study, two groups, each of 50 male and 50 female Swiss SPF mice were fed 0.5 or 0.2% saccharin made by the Remsen-Fahlberg method (Bayer Farma NV, The Netherlands; containing 0.5% *ortho*-toluenesulphonamide) for up to 21 months. A concurrent control group of 50 males and 50 females received a standard diet. At 18 months, 62, 64 and 66 animals were still alive in the groups receiving 0.5 and 0.2% saccharin and in the control group, respectively. One control female developed an anaplastic carcinoma of the bladder, and one male in the 0.2% saccharin group had a noninvasive transitional-cell carcinoma of the bladder (Kroes *et al.*, 1977) (see also 'multigeneration exposure', p. 137).

[1] The Working Group was aware of completed but unpublished studies on the intragastric administration and feeding of saccharin in the diet to mice; studies in progress on the administration in the diet and drinking-water of sodium saccharin to mice; planned studies on the feeding of sodium saccharin to rats; and completed but as yet unpublished studies on the feeding of sodium saccharin to rats (IARC, 1979).

Groups of 50 male and 50 female dde mice were fed saccharin made by the Remsen-Fahlberg method (purity unspecified) at levels of 0, 0.2, 1.0 or 5% for 21 months. No significant difference in tumour incidence was observed between the treated and untreated groups (National Institute of Hygienic Sciences, 1973).

Groups of 25 male and female Charles River CD mice received sodium saccharin (Merck Co. Ltd, USA & Monsanto Industrial Chemicals Co., USA) in the diet at levels of 0, 1 or 5% for up to 2 years. The Monsanto product contained 345 mg/kg *ortho*-toluenesulphonamide. Animals that died before 6 months were not examined, and survival times were not reported. Animals were sacrificed when obvious tumours were seen or when they were moribund; all survivors were killed at 2 years. All animals that survived 6 months or longer were examined grossly, and any tissues with abnormal changes were examined histologically; in addition, all vital organs from at least 12 animals in each group were examined histologically. Vascular tumours were seen with increased frequency in the experimental groups, while lung tumours, hepatomas and lymphomas occurred with apparently equal incidence in control and experimental groups. Any differences in incidence of tumours were not considered to be significant and were reported to be absent in a duplicate experiment; however, no data on the duplicate study were given (Homburger, 1978) [The Working Group noted the inadequate reporting of the experiment].

Rat: Groups of 10 male and 10 female Osborne-Mendel rats received 0, 1 and 5% saccharin (source and purity unspecified) in the diet for up to 2 years. Mortality in pooled controls was 14% at 1 year and 68% at 2 years; surviving animals at 1 year were 7 males and 9 females in the 0 dose group, 10 males and 10 females in the 1% group and 9 males and 9 females in the 5% group; no data were given for 2-year survival rates. Seven/18 animals (sex not specified) in the 5% group developed abdominal lymphosarcomas; 4 of the 7 also had thoracic lymphosarcomas. Urinary bladders were not examined (Fitzhugh *et al.*, 1951) [The Working Group noted the small number of animals in each group].

Groups of 20 male and 20 female Boots-Wistar rats were fed 0, 0.005%, 0.05% or 5% saccharin made by the Remsen-Fahlberg method (Boots & Co., UK; purity unspecified) for 2 years. At 18 months, 15 male and 14 female controls, and 10 male and 10 female rats at the highest dose level were still alive. No statistically significant differences in tumour incidence were found between treated and control animals. Only five bladders, all from animals in the highest dose group, were examined histologically. Urothelial hyperplasia was found in 1 male and 1 female, and a bladder papilloma was found in another female. Bladder parasites were not found (Lessel, 1971).

Groups of 52 male and 52 female BD rats were fed 0, 0.2 or 0.5% sodium saccharin made by the Remsen-Fahlberg method (Bayer-Werken AG, FRG; purity unspecified) for up to 30 months, starting between 70 and 90 days of age (average total doses, 0, 83 and 210 g/kg bw). Survival at 18 months was 55/104 controls, 50/104 animals treated with 0.2%

saccharin and 41/104 animals treated with 0.5% saccharin; at 24 months survival was 6/104, 3/104 and 5/104, respectively. Sixteen percent of all animals had parasites (*Strongyloides capillaria*) in the urinary tract. Benign and malignant mesenchymal tumours were found with a similar frequency in all groups. No bladder tumours were observed (Schmähl, 1973).

Groups of 60 male and 60 female Charles River CD rats were fed diets containing sodium saccharin made by the Remsen-Fahlberg method (Daiwa Chemical Co. Ltd, Japan; purity conformed to USP, BP & FCC specifications) for 26 months, to give daily intakes of 0, 0.09, 0.27, 0.81 or 2.43 g/kg bw. Saccharin treatment did not affect survival of female rats: at 18 months, approximately 50% of the original animals were alive. In male rats, survival was affected in a dose-related manner: thus, at 18 months about 80% of male control rats were alive, but only about 50% of those in the highest dose group survived. By 24 months about 10% of the animals were alive in all groups. A total of 4 transitional-cell tumours of the bladder were found, one in a male and one in a female given 0.09 g/kg bw and two in males fed 0.81 g/kg bw; an angiosarcoma of the bladder was found in a male control. Bladder calculi were recorded, but there was no association between the presence of calculi, saccharin treatment and/or bladder tumours. The animals were free from bladder parasites. The combined incidences of lymphomas and leukaemias was 7/54 in males at the highest dose of saccharin and 2/57 in untreated male controls (Munro *et al.*, 1975).

It was reported in an abstract that groups of 54-56 male Wistar rats were fed 0 or 2.5 g/kg bw per day sodium saccharin (source and purity unspecified) for up to 28 months. Ten to 16 rats of each group were killed at 12 months, 11 of each group at 24 months and all survivors (number unspecified) at 28 months. No urinary bladder tumours were observed (Furuya *et al.*, 1975) [The Working Group noted the incomplete reporting of this experiment].

Groups of Charles River CD male and female rats of unspecified size received saccharin (source and purity unspecified) by an unspecified route (in the diet; or by gastric intubation thrice weekly) for 18 months, followed by a 6-month period of observation. A high incidence of benign tumours of the pituitary and mammary glands was found in surviving controls and experimental animals. Survival times, types of pathological examination, tumour types and other important experimental details were omitted (Ulland *et al.*, 1973) [The Working Group noted the inadequacy of this experiment].

Groups of 25 male Charles River CD-1 rats received sodium saccharin (Merck Co. Ltd, USA & Monsanto Industrial Chemicals Co., USA) in the diet at levels of 0, 1 or 5% for up to 2 years. The Monsanto product contained 345 mg/kg *ortho*-toluenesulphonamide. Animals that died before 6 months were not examined, and survival times were not reported. Animals were sacrificed when obvious tumours were seen or when they were moribund; all survivors were killed at 2 years. All animals that survived 6 months or longer were examined grossly,

and any tissues with abnormal changes were examined histologically; in addition, all vital organs from at least 12 animals in each group were examined histologically. Tumours of the urinary bladder, pituitary, breast and subcutaneous tissue were seen with equal incidence in all groups (Homburger, 1978) [The Working Group noted the inadequate reporting of the experiment]. Ova consistent with the presence of *Trichosomoides crassicauda* were found in approximately one third of all urines examined from animals in the above experiment. Their presence was not correlated with the occurrence of bladder lesions (Bio-Research Consultants, Inc., 1973).

A group of 75 male and 50 female Wistar SPF rats received sodium saccharin made by the Remsen-Fahlberg method and containing 698 mg/kg *ortho*-toluenesulphonamide (Boots & Co., UK) in the drinking-water to give a daily intake of 2 g/kg bw saccharin. Another group of 75 male and 75 females received 4 g/kg bw per day saccharin in the diet. Controls were 55 males and 50 females. The males receiving saccharin in the drinking-water were also given 1% ammonium chloride for 4 weeks then 0.5% for life, in order to correct a treatment-associated rise in urinary pH. Of the male controls, 25 were given ammonium chloride at the same concentrations. No treatment-associated change in urinary pH occurred in either of the treated groups of females or in males receiving saccharin in the diet. The experiment was terminated after 2 years. Survival at 18 months was 49/55 male and 43/50 female untreated controls, 65/75 males and 44/50 females that received saccharin in the drinking-water, and 55/75 males and 52/75 females fed saccharin in the diet. At 2 years, 37/55 male and 13/50 female controls, 49/75 males and 29/50 females receiving saccharin in the drinking-water, and 12/75 males and 16/75 females fed saccharin in the diet were still alive. In control animals, the total tumour incidence was 1/52 in males and 9/46 in females. In rats receiving saccharin in the drinking-water (2 g/kg bw/day), incidence was 11/71 in males and 10/44 in females; while in rats fed saccharin (4 g/kg bw/day), it was 10/70 in males and 7/68 in females. Transitional-cell carcinomas of the urothelium were not seen in male or female controls, but accounted for 1/71 in males (in the ureter) and 1/44 in females (in the renal pelvis) in rats receiving saccharin in the drinking-water, and 3/70 in males (all in the bladder) and 0/68 in females in the saccharin-fed group. The incidence of lymphosarcomas and/or leukaemia was 0/52 in males and 0/46 in female controls, 4/71 in males and 1/44 in females given saccharin in the drinking-water, and 2/70 in male and 1/68 in female saccharin-fed rats. One Leydig-cell tumour was found in each of the saccharin-treated groups of males, but none occurred in the testes of untreated male controls. There was a treatment-associated increase in microcalculi within the renal tubules of male (but not female) saccharin-treated rats, with an incidence of 2/52 in controls, 30/71 in males given saccharin in the drinking-water and 16/70 in saccharin-fed males. The animals were free from bladder parasites (Chowaniec & Hicks, 1979).

Groups of 50 male and 50 female 30-day old Charles-River CD rats were fed either a control diet or a diet containing 5% sodium saccharin prepared by the Maumee process (The Sherwin-Williams Co., USA) and free of *ortho*-toluenesulphonamide. Survival was not affected by treatment. Bladder tumours (benign and malignant) were observed in 1/36 control males and in 7/38 male rats fed saccharin which survived 87 weeks or more (the time

at which the first tumour was observed (P=>0.05). In addition, 1 treated male and 2 treated females had urothelial tumours of the kidney pelvis and 1 treated male had a urethral tumour; no other urothelial tumours were observed in controls. The incidence of bladder calculi was not related to treatment or to tumour incidence. The animals were free of bladder parasites (Arnold *et al.*, 1977, 1980) [The experiment was part of a two-generation study, see p. 138].

A group of 50 female Wistar rats were given 2.0 g/kg bw per day sodium saccharin made by the Maumee process (The Sherwin-Williams Co., USA) in the diet for 2 years. A group of 63 animals served as controls. At week 84, 50/63 controls and 37/50 saccharin-fed rats were still alive. Overall tumour incidences were similar in the two groups; no bladder neoplasms occurred in either group. Mild focal urothelial hyperplasia was seen in one rat fed saccharin. The animals were free from bladder parasites (Hooson *et al.*, 1980) [The Working Group noted that the animals were not started on the test at weaning but had been fed a normal diet for several weeks prior to the start of the study].

Hamster: Groups of 30 male and 30 female random-bred Syrian golden hamsters received saccharin made by the Maumee process (Sigma Chemical Co., USA) at levels of 0, 0.156, 0.312, 0.625 and 1.25% in drinking-water for their natural lifespan. The highest dose level used in this study was the maximum tolerated dose as determined in an 8-week study. The average daily consumption ranged from 44 mg/animal given the 0.156% level to 353 mg/animal given the 1.25% level. The mean survival time was 50-60 weeks in all groups. Pathological changes as well as distribution and histological types of neoplasms were within the range of tumours that occur commonly in hamsters in this colony (Althoff *et al.*, 1975).

Monkey: In an abstract, it was reported that sodium saccharin made by the Remsen-Fahlberg method (Squibb Co., USA, containing 2.4 mg/kg *ortho*-toluenesulphonamide; and Pfaltz & Bauer, Inc., USA, containing 3.2 mg/kg *ortho*-toluenesulphonamide) (Coulston *et al.*, 1975) was given orally at doses of 20, 100 or 500 mg/kg bw per day on 6 days a week to groups of 2, 2 and 3 *Macaca mulatta* (rhesus) monkeys of each sex, respectively. Three animals of each sex served as controls. After 79 months on this regime, 6 male and 6 female monkeys remained in the treated groups; at this time all remaining monkeys were autopsied. Histopathological examination revealed no abnormal pathology in the urinary bladder, kidneys or testis in those surviving the treatment or in those that died during the test (McChesney *et al.*, 1977).

In a study in progress, now in its ninth year, 10 monkeys of 4 different strains are fed 25 mg/kg bw per day sodium saccharin (Fisher Scientific, USA; 'purified'; method of manufacture unspecified) on 5 days per week. Clinical observation has failed to demonstrate any evidence of gross neoplasia; none of the animals have died (Sieber & Adamson, 1978) [The Working Group noted the fact that this study is not yet completed].

Multigeneration exposure

In these studies, animals of each sex of the parent (Fo) generation were fed saccharin from weaning (or very soon after weaning) throughout both pregnancy and the preweaning of their offspring. The offspring were placed on the same diet as their parents for their entire lifespan; thus, their exposure to saccharin was increased by comparison with that of the Fo generation, by the length of the gestation and suckling periods.

Mouse: Saccharin containing 0.5% *ortho*-toluenesulphonamide (Bayer Farma NV, The Netherlands) was fed to groups of Swiss SPF mice in a multigeneration study for life at levels of 0, 0.2 and 0.5% in the diet. The F_0, F3b and F6a generations, consisting of 50 males and 50 females, were used to test the compound for carcinogenicity. The experiments were terminated at 21 months. The survival rates at 18 months were: 66, 62, 64 (F_0: control, 0.5%, 0.2%); 61, 54, 53 (F3b: control, 0.5%, 0.2%); and 67, 48, 54 (F6a: control, 0.5%, 0.2%). Histopathological examination showed that pathological alterations were equally distributed throughout the control and experimental groups. Two male mice, one of the F_0 generation receiving 0.2% saccharin and one of the F3b generation receiving 0.5% saccharin, developed transitional-cell carcinomas of the bladder at 20.5 months. One female control mouse of the F_0 generation had an anaplastic carcinoma of the bladder at 20.5 months (Kroes *et al.*, 1977).

Rat: Groups of 20 male and 20 female weanling Sprague-Dawley rats of the F_1 generation were fed sodium saccharin made by the Remsen-Fahlberg method (source unspecified) at levels of 0, 0.05, 0.5 and 5% of the basal diet for up to 100 weeks. Of F_1 males, 12, 10, 11 and 15 in the respective dosage groups survived to 80 weeks, by comparison with 16, 14, 14 and 19 F_1 females. Seven transitional-cell carcinomas of the urinary bladder developed, all in F_1 males on the 5% saccharin diet (P=0.001). The presence or absence of bladder parasites was not recorded. The total numbers of tumour-bearing animals were: at 0%, 2 males and 8 females; at 0.05%, 1 male and 6 females; at 0.5%, 1 male and 5 females; and at 5.0%, 7 males and 13 females (Tisdel *et al.*, 1974).

Groups of 48 male and 48 female Charles River CD rats of the F_1 generation were fed dietary levels of 0, 0.01, 0.1, 1.0, 5.0 or 7.5% sodium saccharin (method of production and source unspecified) for 28 months. Their parents had been fed the same diet from weaning. There were no significant differences in survival between treated and control animals. Although no difference in bladder cancer incidence was found between F_1 males fed 5% saccharin (1/21) and the F_1 controls (1/25) surviving beyond 18 months, 6/23 F_1 male rats fed 7.5% saccharin developed transitional-cell carcinomas of the bladder. This result was significantly different from that in controls [P=0.018]. There was no apparent correlation between tumour incidence and presence of bladder stones. The bladders were reported to be 'free of visible parasites' (Taylor & Friedman, 1974; US Department of Health, Education, & Welfare, 1973a, b)

Groups of 50 male and 50 female 30-day old Sprague-Dawley rats were fed either a control diet or a diet containing 5% sodium saccharin continuously for life. The saccharin was prepared by the Maumee process (The Sherwin-Williams Co., USA) and was free of *ortho*-toluenesulphonamide. After 3 months on test the animals were mated on a one-to-one basis. All litters were culled to 8 pups (4 males and 4 females) 4 days post-partum in a random manner. The pups were weaned onto their parents' diet, and 50 males and 50 females from each group were randomly selected to constitute the second generation. Survival in the offspring (F_1 generation) was not affected by treatment. Of the F_1 generation animals surviving 67 weeks or longer, at which time the first tumour was observed, none of the 42 male controls but 12 of the 45 saccharin-treated males had developed bladder cancer [P=0.002]. In addition, 1 male had a urethral tumour, and 2 of the 49 surviving females fed 5% sodium saccharin also had bladder cancers. Although urinary bladder calculi were noted occasionally, the incidence of these calculi was not related to the saccharin treatment nor were they associated with the tumours. The animals were free of bladder parasites (see also p.136)(Arnold *et al.*, 1977, 1980).

(b) Skin application

Mouse: A total dose of 0.24 g saccharin, made by the Remsen-Fahlberg method (British Drug Houses, UK) as an 8% solution in acetone was applied thrice weekly to the skin of 'S' strain mice. Twenty-five days after starting the treatment, the animals were given 18 weekly applications of 0.17% croton oil in acetone. At the end of the croton-oil treatment, a total of 14 skin tumours were observed in 7 of the 20 saccharin-treated animals, by comparison with 4 papillomas in 4 of 19 controls treated with croton oil only. The increase was not statistically significant (Salaman & Roe, 1956).

(c) Intraperitoneal administration

Mouse: In a test system designed as a short-term whole animal bioassay in which the development of lung tumours was used as an indication of carcinogenicity, groups of 20 female A/He mice were injected intraperitoneally with 0.1 ml of saccharin (Monsanto Industrial Chemicals Co., USA; purity unspecified) in water, three times a week for 8 weeks. Two dose levels were used, to give total doses of 78 g/kg bw (approx. 3.3 g/kg bw per day) and 15.6 g/kg bw (approx. 0.6 g/kg bw per day). The experiment was terminated after 21 weeks. As controls, 30 females were given water intraperitoneally three times a week for 8 weeks and killed after 24 weeks. Saccharin was negative as assessed by the pulmonary tumour response (Stoner *et al.*, 1973) [The Working Group noted the limitations of a negative result obtained from this test system, see General Remarks on the Substances Considered, Vol. 20, p. 34].

(d) Other experimental systems

Bladder insertion (implantation): Saccharin (source and purity unspecified) (2 mg) was mixed with 4 times its weight of cholesterol. Pellets (9 - 11 mg) containing saccharin were then inserted into the urinary bladder lumina of 20 'stock' *mice* (sex and age unspecified).

An identical group composed of 28 mice received 9-11 mg pellets of cholesterol. The experiment lasted 52 weeks. Of mice that lived 30 weeks, 4/13 saccharin-treated and 1/24 control animals developed bladder cancer (P=0.01) (Allen *et al.*, 1957).

Sodium saccharin (analytically pure; Abbott Laboratories, USA) (4-5 mg) was mixed with 4 times its weight of cholesterol. Pellets (20-24 mg) containing sodium saccharin were then inserted into the urinary bladder lumina in 2 separate trials using groups each composed of 100 female Swiss *mice* aged 60-90 days. Ninety-nine percent of the sodium saccharin disappeared from the pellet within 1.5 days. Identical groups received 20-24 mg pellets of pure cholesterol. The experiment lasted 56 weeks. Only the bladders of animals surviving more than 25 weeks were examined microscopically. The first urinary bladder carcinoma was seen in a saccharin-treated animal 42 weeks after surgical insertion. The overall incidences of bladder carcinomas were 31/66 (trial 1) and 33/64 (trial 2) in saccharin-treated mice as compared with 8/63 (trial 1) and 5/43 (trial 2) in animals exposed to pure cholesterol pellets ($P<0.001$). The carcinomas in saccharin-exposed mice were more frequently multiple and invasive ($P<0.009$). They were composed of cells with a high mitotic index and exhibited more squamous or glandular metaplasia than was found in tumours in control animals. No other tissues demonstrated a tumour incidence deviant from the rate seen in control mice (Bryan *et al.*, 1970) (cf. sodium cyclamate, p. 74).

(e) Administration in conjunction with known carcinogens

Benzo[a]*pyrene (BP):* Groups of 50 female Swiss *mice* received an initial single gastric instillation of 0.2 ml polyethylene glycol either alone or containing 50 μg BP (purities unspecified). Seven days later, the test diet, containing 5% saccharin (British Saccharin Sales Co. Ltd, UK; purity unspecified) was fed for 72 weeks. Average survival rates were not different from those in controls. Although mice treated with BP showed an increased incidence of tumours of the forestomach (20/61), saccharin did not enhance the occurrence (10/32). Hepatocellular adenomas, pulmonary neoplasms and malignant lymphomas occurred with similar frequencies in all groups. No pathological alterations were observed macroscopically in the urinary bladder (Roe *et al.*, 1970) [The Working Group noted that BP is not organotropic for the bladder and that the urinary bladders were not examined histologically].

2-Acetylaminofluorene (AAF): Two groups of 12 Horton Sprague-Dawley female *rats* were fed a diet supplemented with 300 mg AAF/kg of diet for 40 weeks. The test group received in addition 5% sodium saccharin (Abbott Laboratories, USA) in the diet. Eleven of the 12 AAF-fed controls developed palpable mammary and ear-duct tumours in the 40-week period, compared with 6/12 rats fed AAF plus saccharin. In addition, liver tumours were observed in both groups but they were smaller and less malignant in the saccharin-fed animals. Microscopic examination of the urinary bladders indicated that the mucosal lining

was hyperplastic in all rats fed AAF and was particularly so in those fed AAF plus saccharin; one animal in the test groups exhibited squamous metaplasia and precancerous changes of the mucosal epithelium. No malignant lesions of the urinary bladder were observed in any of the rats (Ershoff & Bajwa, 1974) [The Working Group noted the inadequate number of animals and the fact that food consumption was not measured, so that it was not possible to assess the intake of AAF or saccharin].

N-*Nitroso*-N-*methylurea (NMU):* A group of 50 female Wistar SPF *rats*, 6-8 weeks of age, were pretreated with 1.5 mg NMU, then 2 days later were administered 4 g/kg bw per day sodium saccharin (Boots Co., UK; Remsen-Fahlberg, containing an average of 698 mg/kg *ortho*-toluenesulphonamide) in the drinking-water for life or up to 2 years; 50 further females were pretreated with 2 mg NMU and then fed 2 g sodium saccharin/kg bw per day in the diet. NMU (purity unspecified) was dissolved in 0.9% sodium chloride (pH 7.0) and instilled into the bladder. Control groups consisted of 55 male and 50 female untreated rats, 75 males and 50 females given 2 g sodium saccharin /kg bw per day in drinking-water and 75 males and 75 females fed 4 g sodium saccharin/kg bw per day in the diet. For concurrent NMU controls, 85 males and females were given 1.5 mg NMU, and 50 were given 2 mg NMU and maintained on a saccharin-free diet for 2 years. The incidences of transitional-cell neoplasms of the bladder in surviving animals whose bladders were examined histologically were: untreated controls, 0/52 males and 0/46 females; lower dose of sodium saccharin alone in drinking-water, 0/71 males and 0/44 females; higher dose of sodium saccharin alone in diet, 3/70 males and 0/68 females; NMU-treated males and females (1.5 and 2.0 mg) 0/124; NMU followed by the lower dose of sodium saccharin in drinking-water, 23/49 females (47%; $P<0.0005$); NMU followed by the higher dose of sodium saccharin in the diet, 27/47 females (52%; $P<0.0005$). The first bladder tumour was seen after 95 weeks in the saccharin-fed control group and after 8 weeks in the NMU-initiated and saccharin-treated test groups. The animals were free from bladder parasites (Chowaniec & Hicks, 1979; Hicks *et al.*, 1978).

A single dose of 2 mg NMU (German Cancer Research Center, FRG) was instilled into the urinary bladder of female Wistar rats (AF-Han strain) (weighing 195 g). Thereafter, 50 animals were given 2% saccharin (The Sherwin-Williams Co., USA; purity unspecified) in the diet, increased after 10 weeks to 4%, for life (1.4-2.5 g/kg bw per day). Control groups consisted of 100 untreated female rats, 50 females receiving NMU alone and 50 females receiving distilled water. A further group of 50 female rats treated with NMU were given 3% calcium carbonate in the diet instead of saccharin. Survival at two years was: controls, 59/100; water controls, 28/50; NMU-treated, 13/50; NMU + calcium carbonate-treated, 15/50; and NMU + saccharin-treated, 14/50. In the NMU-treated groups, the first tumour of the urinary bladder was found after 14 weeks. Urethelial neoplasms (benign and malignant) occurred in the renal pelvis, ureter and urinary bladder. The overall incidences of urinary tract tumours were 57% (NMU only; survival 76 ± 29 weeks), 65% (NMU + saccharin; survival, 78 ± 25 weeks) and 65% (NMU + calcium carbonate; survival, 86 ± 23 weeks). In the renal pelvis, frequencies were 28, 57 and 43%; the ureter showed incidences

of 17, 12 and 11%; and the urinary bladder had frequencies of 39, 31 and 39%, respectively. Calcifications in the urinary tract, including stone formation, were similar in all treated groups, including water controls; they did not correlate with tumour occurrences. In the untreated controls, as well as in controls receiving a water instillation into the urinary bladder, a tumour of the urinary tract was found. The presence or absence of bladder parasites was not reported (Mohr *et al.*, 1978) [The Working Group noted that many tumours were found and that the animals were heavier than those used in the experiment by Hicks *et al.*, 1978].

Three groups of 63 female Wistar *rats* were pretreated with 0.15 ml of a saturated solution of NMU (purity unspecified) in saline instilled into the bladder. Two weeks later, rats were given 0 or 2.0 g/kg bw per day sodium saccharin in the drinking-water for 2 years ; one group received saccharin prepared by the Maumee process (The Sherwin-Williams Co., USA) and the second group received saccharin prepared by the Remsen-Fahlberg method (Boots Co., UK) containing 40 mg/kg *ortho*-toluenesulphonamide. At week 84, 22 controls, 43 animals given 'Maumee' sodium saccharin, and 37 rats given 'Remsen-Fahlberg' sodium saccharin had died. An increase in the number of proliferative bladder lesions occurred in animals treated with NMU plus saccharin. The incidence of bladder neoplasia was not significantly different in the saccharin-treated groups, but the latent period was shorter (55 and 52 weeks *versus* 87 weeks). The animals were free from bladder parasites (Hooson *et al.*, 1980) [The Working Group noted that the animals were not started on the test at weaning but had been fed a normal diet for several weeks prior to the start of the study].

N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT): Male Fischer *rats*, 4-weeks-old at the start of the experiment, were treated as follows: Group 1, 0.2% FANFT (Sober Laboratories, USA) in powdered diet for 6 weeks, followed immediately by 5% sodium saccharin (Sigma Chemical Co., USA; containing <0.03 mg/kg *ortho*-toluenesulphonamide) in powdered diet for 83 weeks, then standard diet; Group 2, pretreatment with FANFT as Group 1, followed by 6 weeks on standard diet, then 5% sodium saccharin diet for 77 weeks, then standard diet; Group 3, normal diet for 6 weeks, followed by 5% sodium saccharin diet for 83 weeks, then standard diet; Group 4, pretreatment with FANFT as Group 1, followed by standard diet for 98 weeks; and Group 5, untreated controls fed standard diet for 104 weeks. Each group consisted of 20 animals, apart from the control group which had 42 animals. The experiment was terminated after 104 weeks, at which time 6/20, 9/20, 19/20, 16/20 and 27/42 animals survived in groups 1-5, respectively. The incidences of urothelial carcinomas were 18/19 and 13/18 in the FANFT plus saccharin groups, 0/20 in the group receiving saccharin alone, 4/20 in the group receiving FANFT alone, and 0/42 in the untreated controls. In addition, 1/19 animals in Group 1 and 1/18 in Group 2 (FANFT plus saccharin-treated animals) had urinary bladder sarcomas, and 1/20 in Group 4 (FANFT only) had a bladder papilloma. The presence or absence of bladder parasites was not reported (Cohen *et al.*, 1979).

SACCHARIN/CYCLAMATE MIXTURES

(a) Oral administration

Single-generation exposure

Rat: Two groups of 52 male and 52 female Sprague-Dawley rats, between 70 and 90 days of age, were given a 10:1 mixture of sodium cyclamate:sodium saccharin (Bayer-Werken AG, FRG) daily in the diet for up to 30 months. The cyclamate in the mixture contained less than 4 mg/kg cyclohexylamine; no information on the purity of the saccharin was given. The mixture was administered at doses of 2 and 5%. An identical group served as controls. At 24 months, approximately 10% of the initial number of animals were still alive. Except for the occurrence of bladder parasites (*Strongyloides capillaria*) in 16% of animals, all examinations were negative. A similar frequency of benign neoplasms occurred in all groups (fibromas, fibroadenomas or adenomas of the mammary gland in females and thymomas in males) (Schmähl, 1973).

It was reported in an abstract that 2 groups of 54-56 Wistar rats received 0 or 2.5 g/kg bw per day of a mixture of sodium cyclamate:sodium saccharin (10:1) (source and purity unspecified) in the diet for 28 months. Ten to 16 rats of each group were killed at 12 months, 11 at 24 months and all survivors at 28 months. No treated or control animals developed tumours of the urinary bladder (Furuya *et al.*, 1975) [The Working Group noted the incomplete reporting of this experiment].

Groups of 35 male and 45 female FDRL strain Wistar-derived weanling rats were fed a 10:1 mixture of sodium cyclamate:saccharin (Abbott Laboratories, USA; purity and method of manufacture unspecified) in the diet at doses of 0, 500, 1120 and 2500 mg/kg bw per day for 2 years. From week 79 the original dose groups were split, and 50% of the survivors in each group, except the untreated controls, received in addition cyclohexylamine hydrochloride in the diet. The 500 mg group received 25 mg, the 1120 mg group 56 mg, and the 2500 mg group received 125 mg cyclohexylamine/kg bw per day. Mortality rates were similar in control and test groups. Treatment-related pathological changes were seen only in the kidney and bladder. Pelvic hyperplasia was observed more often in the treated groups (8/80, 21/80 and 16/80, as compared with 3/80 in controls). Among animals surviving more than 49 weeks, 9/25 male and 3/35 female rats at the 2500 mg/kg bw dose, compared with 0/35 male and 0/45 female controls, developed transitional-cell carcinomas of the urinary bladder. Of these, 3 male and 2 female rats had received cyclohexylamine. Two of the bladder carcinoma-bearing animals had calculi; 18 rats at this dose level had nonmalignant proliferative bladder lesions. In the lower dose groups, nonmalignant proliferative lesions were found, but their incidence was not significantly higher than that in controls. Renal calcification was seen in 7/12 rats with bladder carcinomas; *Trichosomoides crassicauda* infection was present in one rat with bladder cancer and 4 rats with non-neoplastic proliferative lesions at the highest dose level, in 4 given the 1120 mg/kg dose, in 2 given the 500 mg/kg dose and in 5 control animals (Oser *et al.*, 1975; Price *et al.*, 1970).

Multigeneration exposure

Mouse: In a multigeneration study, a 10:1 mixture of sodium cyclamate:saccharin (5 or 2% and 0.5 or 0.2%, respectively; Bayer Farma NV, The Netherlands) was fed continuously to Swiss SPF mice over 6 generations. The saccharin contained 0.5% *ortho*-toluenesulphonamide; the cyclamate contained 2.1 mg/kg cyclohexylamine. The F_0 (parental) , F3b and F6a generations, consisting of 50 males and 50 females each, were used for the carcinogenicity studies and were treated for 84 weeks. Pathological alterations and urinary bladder calculi occurred with similar frequencies in control and treated groups. Four neoplasms of the urinary bladder occurred: three anaplastic carcinomas (1 in a female control of the F_0 generation and 2 in females of the F_0 and F6a generations fed 0.2% saccharin plus 2% cyclamate) and one papilloma (in a male of the F6a generation given 0.2% saccharin and 2% cyclamate). The mean latent period was more than 80 weeks (Kroes *et al.*, 1977).

ORTHO-TOLUENESULPHONAMIDE

(a) Oral administration

Rat: In a two-generation study, groups of Charles River CD rats (30 days old) were fed one of the following diets with tap-water *ad libitum:* control; 2.5, 25 or 250 mg/kg bw per day *ortho*-toluenesulphonamide; or 250 mg/kg bw per day *ortho*-toluenesulphonamide with 1% ammonium chloride in the drinking-water. The *ortho*-toluenesulphonamide (Monsanto Industrial Chemicals Co., USA) was more than 99.9% pure. Each group contained 50 males and 50 females, except for the group receiving ammonium chloride in the drinking-water, which comprised 40 males and 38 females. The F_0 animals were started on test at 32 days of age. After 3 months on test, the animals were mated on a one-to-one basis; all litters were culled to 8 pups (4 males and 4 females) 4 days post partum in a random manner. The pups were weaned onto their parents' diet, and 50 males and 50 females from each group were randomly selected to constitute the second generation (F_1). The two generations remained on test for 30 (F_1) and 32 (F_0) months. The animals were free of bladder parasites. Rats from both generations fed diets providing 250 mg/kg bw or 250 mg/kg bw plus 1% ammonium chloride in the drinking-water had lowered feed consumption. There were no treatment-related effects associated with longevity. The numbers of bladder tumours (all of which were benign) were: in the F_0 generation males - 1 in a control and 1 in each of the 2.5 and 250 mg/kg bw per day *ortho*-toluenesulphonamide groups; females - 1 in the 2.5 mg/kg bw group; in the F_1 generation females - 2 in the 2.5 mg/kg bw group (Arnold *et al.*, 1977, 1980).

Groups of 38 male and 38 female Sprague-Dawley rats, 3 months of age, were administered daily doses of 0, 20 or 200 mg/kg bw *ortho*-toluenesulphonamide (source and purity unspecified) for lifetime by adjusting concentrations added to the diet. Average survivals were 700 days for controls, 770 days for low-dose and 840 days for high-dose animals. The total incidences of malignant tumours were no different in treated groups compared with

controls. Lymphosarcomas developed in 7/71 controls, 10/75 low-dose and 10/76 high-dose animals. In addition, 3/76 leukoses occurred at the high dose and 5/75 at the low dose, compared with 0/71 in controls. In high-dose animals, 1/76 carcinoma and 4/76 papillomas of the bladder were found after 759-996 days [P=0.03]; in low-dose rats, 3/75 papillomas of the bladder occurred after 539, 766 and 873 days. No bladder tumours occurred in 71 controls (Schmähl, 1978) [The presence or absence of bladder parasites was not recorded and the sexes of animals with bladder tumours were not specified].

Three groups of 50 or 63 female Wistar rats were administered *ortho*-toluenesulphonamide (Monsanto Industrial Chemicals Co., USA; pure) at levels of 0 or 0.1% in the drinking-water or 90 mg/kg in the diet for 2 years. Survival was similar in all groups at 84 weeks. No difference in overall tumour incidence was observed between control and test groups. No bladder tumours were observed in any group. Mild diffuse urothelial hyperplasia was found in 1/50 rats fed *ortho*-toluenesulphonamide in the diet (Hooson *et al.*, 1980).

(b) Administration in conjunction with known carcinogens

N-*Nitroso*-N-*methylurea (NMU):* Three groups of 63 female Wistar *rats* were treated with a single intravesicular dose of 0.15 ml of a saturated solution of NMU in saline. Two weeks later, *ortho*-toluenesulphonamide (Monsanto Industrial Chemicals Co., USA; pure) was administered at levels of 0, 0.08 mg/kg bw in the diet or 0.1% in the drinking-water for 2 years. Survival was similar in all groups at 84 weeks. No difference in overall tumour incidence was seen between control and test groups. Neoplasia and hyperplasia of the bladder occurred in 27% and 35%, respectively, of rats in the NMU control group. No statistical increase in bladder neoplasia or hyperplasia was observed in groups given NMU and *ortho*-toluenesulphonamide (Hooson *et al.*, 1980).

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

Saccharin and its salts

The LD_{50} values for sodium saccharin by oral administration are: mice, 17.5 g/kg bw; random-bred rats, 17 g/kg bw; Wistar rats, 14.2 g/kg bw (Taylor *et al.*, 1968); hamster, 8.7 and 7.4 g/kg bw, in males and females, respectively (Althoff *et al.*, 1975). The LD_{50} by i.p. injection is: mice, 6.3 g/kg bw; random-bred rats, 7.1 g/kg bw (Taylor *et al.*, 1968).

Addition of 0.5% sodium saccharin to the diet reduced the growth rate of rats over a 38-day period. The feeding of 0.065 g/kg bw per day sodium saccharin to dogs for 11 months produced no toxic effect other than occasional stool softening (Taylor *et al.*, 1968).

Addition of 2% sodium saccharin to the diet of dogs for 16 weeks or of rats for 13 weeks had no noticeable toxic effects (Kennedy *et al.*, 1976). Hamsters given 1.25% saccharin for 8 weeks in their drinking-water showed no toxic effects (Althoff *et al.*, 1975). Administration of 1% sodium saccharin in drinking-water and/or 5% in food decreased weight gain and caused death in rats fed reduced food rations (Strouthes, 1978).

Administration to rats of 2 g/kg bw per day sodium saccharin in the drinking-water or of 4 g/kg bw per day in the diet reduced weight gain markedly; fluid intake was increased in the latter group and decreased in the former. The urinary pH of males of the first group rose above 7.0 after 27 weeks, and some animals showed marked crystalluria. These pH changes were reversible. The most important treatment-related findings were increased incidences of microcalculi and telangiectasia of the vasa recta in kidneys, of renal pelvic hyperplasia , of extramedullary haematopoiesis and of hepatic zonal necrosis. Hyperplasia of the bladder epithelium occurred earlier in animals of the second group (Chowaniec & Hicks, 1979).

In a six-generation experiment with Swiss mice receiving 0.2 or 0.5% saccharin (containing 0.5% *ortho*-toluenesulphonamide) in their diet, no effect on weight gain and no histopathological alterations due to treatment were found in long-term studies (21 months) performed with the 1st, 3rd and 6th generations (Kroes *et al.*, 1977).

Saccharin is a competitive inhibitor of glucose-6-phosphatase *in vitro* (Lygre, 1974, 1976) and inhibits guanylate cyclase (Vesely & Levey, 1978). It also inhibited the induction of liver tryptophan oxygenase (Sabri *et al.*, 1969).

Chronic feeding of 7.5% sodium saccharin in the diets of rats inhibited epithelial DNA synthesis in the urinary bladder (Lawson, 1978).

ortho-*Toluenesulphonamide*

The LD_{50} by oral administration in rats is about 2 g/kg bw (Schmähl, 1978).

Teratogenicity and embryotoxicity

No effects on reproduction were observed in 20 mice receiving 194 mg/kg bw saccharin daily for 180 days (Lehmann, 1929). Oral doses of up to 600 mg/kg bw per day saccharin or its sodium salt given over the total organogenesis phase have not been found to induce malformations or other embryotoxic effects in mice (Lorke, 1969), rats (Fritz & Hess, 1968; Lessel, 1971) or rabbits (Klotzsche, 1969; Lessel, 1971).

Feeding of male and female mice for 10 weeks with a diet containing 1% sodium saccharin, corresponding to a daily intake of about 2000 mg/kg bw, had no effect on their fertility when subsequently mated and caused no biologically important increase in pre-implantative or post-implantative losses (Lorke & Machemer, 1975).

Multigeneration experiments, including studies on reproductive capacity and perinatal development, and teratological studies, performed with Swiss mice receiving 0.2 or 0.5% saccharin in the diet revealed no pathological effects (Kroes *et al.*, 1977).

Tanaka (1964) reported that saccharin is about 100 times more toxic to fetal than to adult mice; however, these data appear to contradict all other results. Serious doubts have been raised about the validity of Tanaka's data and his experimental approach (Lorke, 1969), and Tanaka *et al.* (1973) could not confirm his earlier findings.

It was reported in an abstract that in a three-generation reproduction study with Charles River CD rats, average weaning weights were decreased compared with controls in litters from parents that received 5 or 7.5% sodium saccharin in the diet, but survival was not affected. Other reproductive indices showed scattered variations, but were not consistent over all generations (Taylor & Friedman, 1974).

It was reported in an abstract that no significant increase in fetal deaths, number of resorptions or drug-induced teratogenic effect was found in hamsters administered 10 and 100 g/day of calcium saccharin or a mixture of calcium saccharin and calcium cyclamate ('Sucaryl') during pregnancy. A decrease in litter size was seen in rats given the highest dose. No decrease in survival from birth to weaning was seen in either hamsters or rats (Adkins *et al.*, 1972).

No evidence of a primary embryotoxic effect was seen in Wistar rats treated with 0.4% sodium saccharin [meeting the standards established in the Federal Republic of Germany (see section 1.4)] for 20 days after mating; histological examination revealed no ocular damage at term or at the age of 3 weeks. Impurities were not tested (Luckhaus & Machemer, 1978).

While the majority of investigators found no abnormalities in animals treated with saccharin during pregnancy, Lederer (1977) and Lederer & Pottier-Arnould (1973) reported morphological changes of the eye lens and increased embryonic mortality in offspring of pregnant Wistar rats fed 0.3 and 3% saccharin in the diet. Lederer (1977) concluded that the anomalies found were due to impurities in commercial saccharin synthesized by the Remsen-Fahlberg method, since the anomalies did not occur in animals treated with saccharin made by the Maumee procedure. The contaminating compounds of the saccharin produced by the Remsen-Fahlberg method, when tested separately, also induced ocular changes. *ortho*-Sulphobenzoic acid was most active when added to feed at a level of 0.1%; *ortho*-sulphamoylbenzoic acid and ammonium-*ortho*-sulphobenzoic acid at dietary levels of 0.1% also

increased the incidence of both ocular abnormalities and mortality over that seen in controls; and *ortho*-toluenesulphonamide was almost inactive. Lederer reported that a few ocular abnormalities also occurred in his controls [The possibility of histological artefacts has not been ruled out]. Lederer & Pottier-Arnould (1969) found an increased mortality in the offspring of mice given 5% saccharin in their diet.

It was reported in an abstract that no toxicological changes were noted in dogs that received 0.5-1.5 g/kg bw per day of a 10:1 combination of sodium cyclamate:sodium saccharin [corresponding to daily doses of 45-140 mg/kg bw sodium saccharin] during pregnancy or in their offspring that received the same dose up to the age of one year (Fancher *et al.*, 1968).

Absorption, distribution, excretion and metabolism

Saccharin

^{14}C-Saccharin administered by i.v. infusion to 5 rhesus monkeys (4 μg/kg bw per min for 60 min) in the last trimester of pregnancy crossed the placenta rapidly and was distributed in all fetal tissues except the central nervous system. At the end of the infusion period, fetal blood levels were approximately 30% of maternal values. In contrast to the maternal organism, in which radioactivity decreased quickly after infusion ended, saccharin cleared very slowly from the fetal compartment, and 2 hours after termination of the infusion, fetal blood levels were higher than maternal ones. The slow rate of fetal clearance suggests that considerable accumulation might result from repetitive maternal ingestion. No data were available on the penetration of saccharin into the embryonic compartment during the organogenesis stage (Pitkin *et al.*, 1971a).

Saccharin was excreted rapidly unchanged by rhesus monkeys (Pitkin *et al.*, 1971b) and by guinea-pigs; about 70% was found in rat urine and the remainder in the faeces (Minegishi *et al.*, 1972). Although it is rapidly excreted by rats, some accumulates in the bladder; but after removal of saccharin from the diet, it is completely cleared within 3 days (Matthews *et al.*, 1973). Lethco & Wallace (1975) also found that the highest levels of radioactivity after administration of ^{14}C-labelled saccharin were in the kidney and bladder and that the metabolic profiles of dogs, rabbits, guinea-pigs and hamsters were similar.

Sodium ^{35}S-saccharin instilled into the bladder of male rats was absorbed into the plasma (Colburn, 1978).

The accumulation of saccharin by rat renal cortical tissue incubated *in vitro* was dependent upon oxygen and was reduced by metabolic inhibitors, suggesting that saccharin is eliminated by active tubular secretion (Goldstein *et al.*, 1978). Saccharin forms ion-pair complexes with bases such as quinine and ephedrine and facilitates the absorption of these bases from the rat rectum (Kakemi *et al.*, 1969).

In many studies on the metabolism of saccharin in several animal species, no metabolites have been detected. Byard & Golberg (1973) showed that 90% of ^{14}C-labelled saccharin was excreted unchanged by rats and monkeys of both sexes. Even after pretreatment with phenobarbitone or sodium saccharin no metabolites of saccharin were found. Ball *et al.* (1977) showed that it was not metabolized by liver microsomal preparations or by faecal homogenates taken from rats fed 1% saccharin in the diet for 2 years. No binding of saccharin to DNA of rat liver and urinary bladder was found 5 hours after oral administration of 372-390 mg/kg bw ^{35}S-saccharin (Lutz & Schlatter, 1977).

ortho-*Toluenesulphonamide and other impurities*

Of the more than 30 impurities that have been identified in commercial saccharin, data on metabolism are available for only a few. The rates at which 7 intragastrically administered impurities of saccharin [radiolabelled *ortho*-toluenesulphonamide, benz(*d*)isothiazoline-1,1-dioxide, 3-aminobenz(*d*)isothiazoline-1,1-dioxide, 5-chlorosaccharin, toluene-4-sulphonamide and 4-sulphamoylbenzoic acid] were eliminated in rats were similar. At doses ranging from 20-80 mg/kg bw, 80-95% of the impurities were recovered within 24 hours in urine and faeces; urinary metabolites of these impurities were identified (Ball *et al.*, 1978; Renwick, 1978; Renwick & Williams, 1978; Renwick *et al.*, 1978).

In female Wistar rats given single oral doses of 20, 125 or 200 mg/kg bw ^{14}C-*ortho*-toluenesulphonamide, 79, 58 and 36% of the activity were recovered in 24-hour urine samples; 24-48-hour elimination was 7, 14 and 33% of the dose, respectively. Within 7 days, 4.5, 5.9 and 7% of the activity was recovered from the faeces. The main metabolites in the urine were 2-sulphamoylbenzyl alcohol and its sulphate or glucuronic acid conjugates (80%), *N*-acetyltoluene-2-sulphonamide (6%), saccharin (3%) and 2-sulphamoylbenzoic acid (2%) (Renwick *et al.*, 1978).

In a similar study, 50% of administered *ortho*- and *para*-toluenesulphonamides excreted in urine had been metabolized to *ortho*- and *para*-sulphamoylbenzoic acids, respectively (Minegishi *et al.*, 1972).

Mutagenicity and other short-term tests

Saccharin

Saccharin of various degrees of purity was not found to be mutagenic in the *Salmonella*/microsome assay when the standard plate method was used (Ashby *et al.*, 1978; McCann, 1977; Pool, 1978; Stoltz *et al.*, 1977).

There is an isolated report that a pharmaceutical preparation of saccharin was weakly mutagenic to TA98 and TA100 strains when a modified plate procedure using reisolated strains of the standard *Salmonella* assay tester strains was followed; the urine of mice given 2.5 g/kg bw pure or impure saccharin orally was also reported to be mutagenic to reisolated strains of TA98 and TA100 with this modified protocol (Batzinger *et al.*, 1977).

Mutagenic effects of both purified and impure lots of sodium saccharin have been observed in mouse lymphoma L5178Y cells in the presence of liver homogenate in a dose range of 10-14 mg/ml. Increases in the frequency of trifluorothymidine-resistant mutants were extremely small, and no clear dose-response effect was obtained (Clive *et al.*, 1979).

A highly purified preparation of saccharin [synthesized by the Maumee process, provided by Dr R. Stoltz, Canada] caused a significant, dose-related increase in chromosome aberrations (breaks, gaps, translocations and ring formations) in Chinese hamster ovary (CHO) cells in the presence of liver homogenate (McCann, 1977). It was reported in an abstract that chromatid breaks and gaps were also induced in CHO-K1 cells treated with sodium saccharin (purity unspecified) (Yoshida *et al.*, 1978). Aberrations have been induced by saccharin and its sodium salt in other Chinese hamster cell lines (Abe & Sasaki, 1977; Ishidate & Odashima, 1977; Kristoffersson, 1972; Masubuchi *et al.*, 1978a).

I.p. injections of 4 g/kg bw sodium saccharin significantly increased chromosome breaks and gaps in bone-marrow cells of male mice (Masubuchi *et al.*, 1978a), but not in those of hamsters given 1.5 g/kg bw saccharin orally for 3 days (van Went-de Vries & Kragten, 1975); Leonard & Leonard (1979) obtained negative results with male C57Bl mice injected intraperitoneally with 4 g/kg bw sodium saccharin. No chromosome damage was detected in spermatogonia of Chinese hamsters given two doses of 5 g/kg bw sodium saccharin orally (Machemer & Lorke, 1975), or in spermatocytes of male C57Bl mice given 20 g saccharin/l drinking-water for 100 days (Leonard & Leonard, 1979).

Sister chromatid exchanges were induced by saccharin and its sodium salt in human (Wolff & Rodin, 1978) and hamster cells (Abe & Sasaki, 1977; Wolff & Rodin, 1978) *in vitro*.

Rao & Qureshi (1972) observed an increase in the number of dominant lethal mutations following administration to 20 male mice of 1.72% sodium saccharin in drinking-water for 30 days. Šrám & Zudová (1974) reported a dose-related increase in the incidence of dominant lethal mutations in mice, with a maximum frequency after 5 i.p. injections of 200 mg/kg bw sodium saccharin. In the same experiment, translocations and other aberrations in spermatocyte chromosomes were induced by this treatment. Masubuchi *et al.* (1978b) found a statistically significant increase in dominant lethal mutations within 2 weeks after treatment in mice given a single i.p. injection of 2 g/kg bw sodium saccharin; fertility of treated animals was low. Oral doses of 5 g/kg bw sodium saccharin given to NMRI mice for 5 days or of 20 g saccharin/l drinking-water given to C57Bl mice for 100 days had no effect on dominant lethality (Machemer & Lorke, 1973).

In several experiments testing saccharin in *Drosophila melanogaster*, no sex-linked recessive lethal mutations were induced (McCann, 1977; Samuel & Rao, 1972); however, the limit of sensitivity in the most extensive of these studies was detection of a 4-fold increase in the incidence of recessive lethal mutations (McCann, 1977). Some batches of commercial saccharin induced recessive lethal mutations in *Drosophila*, while others did not (Kramers, 1977), so that earlier positive results (Šrám & Weidenhofferová, 1969; Šrám & Zudová, 1972) may have been due to contaminants.

It was reported in an abstract that sodium saccharin caused a dose-related increase in unscheduled DNA synthesis in human fibroblasts treated *in vitro* (Ochi & Tonomura, 1978). There was no evidence of mitotic recombination in *Saccharomyces cerevisiae* D3 (McCann, 1977).

An impure sample used in the cancer bioassay of the Health Protection Branch of Canada (Arnold *et al.*, 1977, 1979) and purified samples of saccharin (provided by Dr R. Stoltz, Canada) (2 mg/ml) did not produce oncogenic transformation of C3H/10T½ mouse embryo fibroblasts *in vitro*. However, after treatment of the cells with a nontransforming initiating dose (0.1% μg/ml) of 3-methylcholanthrene, continuous treatment with either sample of saccharin (100 μg/ml) led to significant transformation. In this system saccharin was 1000-fold less active than the tumour promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (Mondal *et al.*, 1978).

ortho-*Toluenesulphonamide and other impurities*

ortho-Toluenesulphonamide was not mutagenic in the *Salmonella*/microsome plate assay using strains TA98, TA100, TA1535, TA1537 and TA1538, with or without Arochlor 1254-induced rat liver 9000 x *g* supernatant. The doses used were up to 1 mg/plate (Jagannath & Brusick, 1978; Stoltz *et al.*, 1977) and 2.5 mg/plate (Ashby *et al.*, 1978). A similar study with negative results was reported by Poncelet *et al.* (1979). Wild *et al.* (1980) obtained a doubling of the mutation rate in TA98 at very high doses (up to 14 mg/plate) in the presence of Arochlor-induced rat-liver 9000 x *g* supernatant and only on a special medium other than the Vogel-Bonner-E-medium. A very similar effect was observed with *para*-toluenesulphonamide.

Impurities extracted with organic solvents from some lots of saccharin were active in tester strains TA98, TA1538 and TA100 (McCann, 1977; Stoltz *et al.*, 1977). *ortho*-Sulphobenzoic acid and ammonium *ortho*-sulphobenzoic acid were not mutagenic in the standard tester strains of *Salmonella typhimurium*, with or without rat liver post-mitochondrial fraction (Poncelet *et al.*, 1979).

Doses of up to 1 mg/plate *ortho*-toluenesulphonamide did not induce gene conversion in *Saccharomyces cerevisiae* strain D4, with or without metabolic activation (Jagannath & Brusick, 1978).

Injection of 0.2 µl or feeding of 5 mM of *ortho*-toluenesulphonamide did not increase the incidence of sex-linked recessive lethal mutations in *Drosophila melanogaster* (Kramers, 1977); however, in a larger-scale study, Wild *et al.* (1980) found a statistically significant doubling of the frequency after 3 days' feeding of a 0.05% solution of *ortho*- or *para*-toluenesulphonamide.

No increase in the number of breaks, gaps and other aberrations was seen in CHO-K1 cells after 24-hours' treatment with 0.9-400 µg/ml *ortho*-toluenesulphonamide (Masubuchi *et al.*, 1977, 1978c).

Concentrations of 0.025-2500 µg/ml *ortho*-toluenesulphonamide produced no morphological transformation in BHK 21/Cl 13 cells (Ashby *et al.*, 1978). Oral and i.p. doses of up to 2 x 1 g/kg bw *ortho*- and *para*- toluenesulphonamide did not induce micronuclei in mouse bone-marrow cells (Wild *et al.*, 1980).

(b) Humans

Saccharin

Doses of more than 3 g saccharin per day cause some disturbances in digestion (Neumann, 1926). No metabolic disturbances were observed in subjects with diabetes mellitus administered 8 g saccharin (Prőls *et al.*, 1973). Allergic reactions to saccharin have been reported (Gordon, 1972; Miller *et al.*, 1974); and Fujita *et al.* (1965) reported 5 patients in whom oral administration of 0.1 g saccharin caused pruritis and oedematous papules on the trunk and limbs.

Saccharin diffuses into lymph, cerebrospinal fluid, saliva, tears and milk (Carlson *et al.*, 1923). About 90% of that present in plasma is bound to serum albumin (Ågren & Bäck, 1973).

In 3 volunteers, 85-92% of doses of 1 g [3-^{14}C]-saccharin administered orally for 21 days was excreted unchanged in the urine within 24 hours; no metabolites were found (Ball *et al.*, 1977). Within 48 hours, 92.3% of a dose of 500 mg ^{14}C-saccharin was excreted in the urine and 5.8% in the faeces (Byard *et al.*, 1974).

Stone *et al.* (1971) studied 975 women delivered of children who were not mentally retarded and women delivered of 247 mentally retarded children. They found that more mothers of children with Down's syndrome and other causes of mental retardation had used artificial sweeteners before and during pregnancy than had controls (Table 4).

Table 4. Mothers of mentally retarded and normal children who had used artificial sweeteners during pregnancy

	Year of delivery		
	1959-61	1962-64	1965-69
Mothers of mentally retarded children	14/79 (17.7%)	26/115 (22.6%)	19 53 (35.8%)
Mothers of normal children	9/78 (11.5%) not significant	35/242 (14.5%) χ^2=4.75, P<0.05	141/655 (21.5%) χ^2=4.96, P<0.05
Relative risk[1]	1.7	1.7	2.0

In addition, the study showed that users of artificial sweeteners had an increased incidence of other adverse outcomes of pregnancy: 'behavioural problems' (incidences - 5.4%, 10/ 185, in children of artificial sweetener users and 2.0%, 15/790, in children of non-users) and 'physical anomalies' (mainly deformities of the bones and joints of the hip, leg and foot; incidences - 4.8%, 9/185, in children of artificial sweetener users and 1.5%, 12/790, in children of non-users [P<0.01]) [Neither age, parity, smoking habits, the presence of diabetes mellitus nor socio-economic status were controlled for in the analysis, and no data were presented on how much artificial sweetener was used. The diverse effects found might argue against a direct effect of the artificial sweeteners, particularly in the absence of a prior expectation that exposure to such agents may cause such abnormalities. Further epidemiological data are needed before concluding that the use of artificial sweeteners in pregnancy is associated with fetal damage].

Kline *et al.* (1978) compared saccharin use in 545 women who had had spontaneous abortions (<28 weeks gestation) and that in 308 women delivering after 28 weeks. Cases were matched with controls within 2 years of age at last menstrual period. There were no statistically significant differences between cases and controls with respect to language spoken at interview, marital status, ethnic group or education level of patient or husband. Occupational level and mean income were slightly greater in cases, and controls more often reported welfare as the principal source of income [These last two factors were not controlled for in the analysis]. Age at last menstrual period, number of previous abortions, smoking and obesity were controlled for using a multiple logistic regression analysis, and diabetics and suspected diabetics were excluded. Saccharin was used by 30 cases (5.5%) and by 18 controls (5.8%) (relative risk, 0.94; 95% confidence interval, 0.5-1.8) [Information

[1] Calculated by the Working Group

on the intake of saccharin did not include use of presweetened drinks and food. No data were available on whether saccharin was used before or during pregnancy, or both, and no information on dose was presented. Most chromosomally abnormal conceptions are lost quite early in pregnancy (often before a women may even be aware that she is pregnant), and, as the authors point out, few women aborting for this reason will be included in a hospital series. This factor cannot be evaluated fully, since the gestational ages of the cases are not reported].

ortho-*Toluenesulphonamide*

Low oral doses of 0.2-0.4 mg/kg bw ^{14}C-*ortho*-toluenesulphonamide were excreted more slowly in humans than in rats, with about 50% of the activity being excreted in the urine within 24 hours and 80% within 48 hours. Less than 1% of the activity was found in faeces. The main urinary metabolites were 2-sulphamoylbenzyl alcohol and its sulphate and glucuronic acid conjugates (35%), saccharin (35%), 2-sulphamoylbenzoic acid (4%) and *N*-acetyltoluene-2-sulphonamide (2%) (Renwick *et al.*, 1978).

3.3 Case reports and epidemiological studies

See **Studies in Humans of Cancer in Relation to the Consumption of Artificial, Non-nutritive Sweetening Agents,** pp. 171-183.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Saccharin has been tested by oral administration in mice, rats and hamsters. In mice, saccharin produced no difference in tumour incidence between treated and control animals in one single and in one multigeneration study. Two further studies by oral administration in mice and three in rats were considered to be inadequate for evaluation. A study in hamsters by oral administration and one study in mice by skin application could not be evaluated. A study in mice by bladder insertion provided evidence for the induction of bladder carcinomas.

Sodium saccharin has been tested by oral administration in mice, rats and monkeys. One study in mice was inadequate for evaluation. One single-generation study in rats showed an increased incidence of bladder tumours in males; two further studies showed a few bladder tumours; one other study showed no difference in tumour incidence between treated and control animals; and two others were inadequate for evaluation. In three two-generation studies in rats, sodium saccharin produced a statistically significant increase in bladder tumours in F_1 males. Sodium saccharin has also been tested in mice by bladder

insertion (implantation): it increased the incidence of bladder carcinomas. It has also been tested by oral administration in monkeys and by intraperitoneal administration in mice, but these experiments were considered to be inadequate for evaluation.

The combination of sodium saccharin with sodium cyclamate in a ratio of 1:10 has been tested by oral administration in a multigeneration experiment in mice and in three single -generation experiments in rats. In one study in rats, transitional-cell carcinomas in the bladder were produced in male animals given the highest dose; in a further study in rats and in the study in mice, there was no difference in tumour incidence between treated and control animals. The other study in rats was inadequate for evaluation.

In one study, female rats were administered sodium saccharin in the drinking-water or diet after receiving a single instillation into the bladder of a low dose of *N*-nitroso-*N*-methylurea (NMU): a high incidence of transitional-cell neoplasms of the bladder was found compared with animals that received NMU alone. Sodium saccharin was also tested in male rats pretreated with *N*-[4(5-nitro-2-furyl)-2-thiazolyl] formamide, resulting in an increased incidence of carcinomas of the bladder over that seen in rats given the latter compound alone.

ortho-Toluenesulphonamide was tested by oral administration in rats in a two-generation study: no increase in bladder tumour incidence was noted in animals of either generation. In one of two single-generation studies in rats, benign and malignant bladder tumours were found.

There is little evidence that saccharin itself induces point mutations. Dominant lethal effects and unscheduled DNA synthesis have been reported; and it causes sister chromatid exchanges and other chromosomal effects.

In the majority of the studies, no indication for a teratogenic effect of saccharin was found; impurities may be responsible for the occasional effects reported. There is no evidence that *ortho*-toluenesulphonamide is mutagenic, although impurities extracted from some lots of saccharin were mutagenic in the *Salmonella*/microsome test. In one *in vitro* test, saccharin was found to enhance the neoplastic transformation of fibroblasts treated with 3-methylcholanthrene.

4.2 Human data

Mortality from bladder cancer has been investigated in two studies by examination of time trends in the United States and in England and Wales. These have shown no marked increase in incidence or mortality from bladder cancer following a substantial increase over a few years in the use of cyclamates and saccharin, but such studies are too insensitive to exclude completely a carcinogenic effect.

In two studies of cancer mortality in patients with diabetes mellitus (who, as a group, have been shown to consume larger quantities of artificial sweeteners than the general population), lower mortality from cancer at all sites was observed as compared with the general population; there was no excess of bladder cancer in particular. In a further study, the frequency of the mention of diabetes mellitus in death certificates of persons who had died of bladder cancers was compared with that in those of controls who had died of other cancers (excluding the lung and pancreas); in the presence of diabetes mellitus, there was no increase in the risk of bladder cancer. As there are differences other than artificial sweetener use between diabetics and the general population, such studies cannot exclude a small carcinogenic effect of these sweeteners.

Seven case-control studies were considered by the Working Group. Only two of these studies examined confounding factors in detail. Of these two, one suggested that use of nine or more tablets of artificial sweeteners per day (or more than eight tablets of saccharin per day) was positively associated with risk for bladder cancer in men but not in women, although in these small groups the results may have been due to chance, to unsuspected confounding factors, or to residual effects of those confounding factors that were considered in the analysis and could be shown to reduce the magnitude of the association. The other study that considered confounding factors suggested that there was no effect of the use of artificial sweeteners on the incidence of bladder cancer; the observed relative risk was 1.0 (indicating no increase in risk), but a relative risk below 1.4 could not be excluded. The other five case-control studies also showed no association, although they were limited by some inadequacies in experimental design.

In six of the seven case-control studies, women with bladder cancer showed a tendency to consume less artificial sweeteners than female controls. This observation suggests that there is no association between use of artifical sweeteners and bladder cancer in women.

4.3 Evaluation [1]

Although a small increase in the risk of urinary bladder cancer in the general population or a larger increase in some individuals consuming very high doses of saccharin and cyclamates cannot be excluded, the epidemiological data provide no clear evidence that saccharin alone, or in combination with cyclamates, causes urinary bladder cancer. There are no epidemiological studies on a possible association between use of saccharin and cyclamates and cancer at other sites in humans.

There is *sufficient evidence* that saccharin alone, given at high doses, produces tumours of the urinary tract in male rats and can promote the action of known carcinogens in the bladder of rats of both sexes; and there is *limited evidence* of its carcinogenicity in mice. There is *limited evidence* that *ortho*-toluenesulphonamide is carcinogenic when given orally to rats; but the available data suggest that impurities at the levels normally found in commercial saccharin do not contribute to the carcinogenicity of saccharin.

[1] See footnote pp. 182-183

5. References

Abe, S. & Sasaki, M. (1977) Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J. natl Cancer Inst., 58*. 1635-1641

Adkins, A., Hupp, E.W. & Gerdes, R.A. (1972) Biological activity of saccharins and cyclamates in golden hamsters (Abstract). *Texas J. Sci., 23*, 575

Ågren, A. & Bäck, T. (1973) Complex formation between macromolecules and drugs. VIII. Binding of saccharine, *N*-methyl saccharine, and the diuretic drugs hydroflumethiazide and bendroflumethiazide to human serum albumin. *Acta pharm. suecica, 10*, 223-228

Allen, M.J., Boyland, E., Dukes, C.E., Horning, E.S. & Watson, J.G. (1957) Cancer of the urinary bladder induced in mice with metabolites of aromatic amines and tryptophan. *Br. J. Cancer, 11*, 212-231

Althoff, J., Cardesa, A., Pour, P. & Shubik, P. (1975) A chronic study of artificial sweeteners in Syrian golden hamsters. *Cancer Lett., 1*, 21-24

Anon. (1963) Maumee moves closer to optimum process to make saccharin. *Chemical Engineering News*, 9 December, pp. 76-78

Anon. (1978) Rules issued on saccharin warning label. *FDA Consumer*, February, pp. 4-5

Arnold, D.L., Moodie, C.A., Stavrić, B., Stoltz, D.R., Grice, H.C. & Munro, I.C. (1977) Canadian saccharin study. *Science, 197*, 320

Arnold, D.L., Moodie, C.A., Grice, H.C., Charbonneau, S.M., Stavrić, B., Collins, B.T., McGuire, P.F. & Munro, I.C. (1980) Long term toxicity of *ortho*toluenesulfonamide and saccharin in the rat. *Toxicol. appl. Pharmacol., 52*, 113-152

Ashby, J., Styles, J.A., Anderson, D. & Paton, D. (1978) Saccharin: an epigenetic carcinogen/mutagen? *Food Cosmet. Toxicol., 16*, 95-103

Ball, L.M., Renwick, A.G. & Williams, R.T. (1977) The fate of [^{14}C]saccharin in man, rat and rabbit and of 2-sulphamoyl[^{14}C]benzoic acid in the rat. *Xenobiotica, 7*, 189-203

Ball, L.M., Williams, R.T. & Renwick, A.G. (1978) The fate of saccharin impurities. The excretion and metabolism of [^{14}C]toluene-4-sulphonamide and 4-sulphamoyl[^{14}C]-benzoic acid in the rat. *Xenobiotica, 8*, 183-190

Batzinger, R.P., Ou, S.-Y.L. & Bueding, E. (1977) Saccharin and other sweeteners: mutagenic properties. *Science, 198*, 944-946

Beck, K.M. (1969) *Sweeteners, nonnutritive.* In: Kirk, R.E. & Othmer, D.F., eds, *Encyclopedia of Chemical Technology,* 2nd ed., Vol. 19, New York, John Wiley & Sons, pp. 593-607

Belcher, R., Bogdanski, S.L., Sheikh, R.A. & Townshend, A. (1976) Determination of saccharin in soft drinks by molecular emission cavity analysis. *Analyst, 101*, 562-565

Bio-Research Consultants, Inc. (1973) *Studies on Saccharin and Cyclamates*, Final Report, Contract NIH-NCI-E-68-1311, Cambridge, MA, National Institutes of Health, Bethesda, MD

Bryan, G.T., Ertürk, E. & Yoshida, O. (1970) Production of urinary bladder carcinomas in mice by sodium saccharin. *Science, 168*, 1238-1240

Bundesminister der Justiz, ed. (1979) *Bundesgesetzblatt*, 4. Anlage 2, liste 12, Bonn, Bundersanzeiger Verlagsges. mbH, pp. 82-83

Byard, J.L. & Golberg, L. (1973) The metabolism of saccharin in laboratory animals. *Food Cosmet. Toxicol., 11*, 391-402

Byard, J.L., McChesney, E.W., Golberg, L. & Coulston, F. (1974) Excretion and metabolism of saccharin in man. II. Studies with ^{14}C-labelled and unlabelled saccharin. *Food Cosmet. Toxicol., 12*, 175-184

Canadian Health & Welfare Department (1977) Canadian position on saccharin. *News Release 1977-40*, 9 March, Ottawa

Carlson, A.J., Eldridge, C.J., Martin, H.P. & Foran, F.L. (1923) Studies on the physiological action of saccharin. *J. Metab. Res., 3*, 451-477

Chowaniec, J. & Hicks, R.M. (1979) Response of the rat to saccharin with particular reference to the urinary bladder. *Br. J. Cancer, 39,* 355-375

Clive, D. Johnson, K.O., Spector, J.F.S., Batson, A.G. & Brown, M.M.M. (1979) Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. *Mutat. Res., 59*, 61-108

Cohen, S.M., Arai, M., Jacobs, J.B. & Friedell, G.H. (1979) Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. *Cancer Res., 39*, 1207-1217

Colburn, W.A. (1978) Absorption of saccharin from rat urinary bladder. *J. pharm. Sci., 67*, 1493

Commission of the European Communities (1978) Commission recommendation of 29 March 1978 to the member states on the use of saccharin as a food ingredient and for sale as such in tablet form to the final consumer. *J. off. Communautés eur., L. 103*, 32

Coulston, F., McChesney, E.W. & Golberg, L. (1975) Long-term administration of artificial sweeteners to the rhesus monkey *(M. mulatta). Food Cosmet. Toxicol., 13,* 297-302

Crammer, B. & Ikan, R. (1977) Properties and syntheses of sweetening agents. *Chem. Soc. Rev., 6*, 431-453

Crampton, R.F. (1975) *The questions of benefits and risks.* In: *Sweeteners, Issues and Uncertainties*, Academy Forum, 4, Washington DC, National Academy of Sciences, pp. 127-132

Eng, M.-Y., Calayan, C. & Talmage, J.M. (1977) Determination of sodium saccharin in chewing gum by high pressure liquid chromatography. *J. Food Sci., 42*, 1060-1061

Ershoff, B. H. & Bajwa, G.S. (1974) Inhibitory effect of sodium cyclamate and sodium saccharin on tumor induction by 2-acetylaminofluorene in rats. *Proc. Soc. exp. Biol. (N.Y.), 145*, 1293-1297

Fancher, O.E., Palazzolo, R.J., Blockus, L., Weinberg, M.S. & Calandra, J.C. (1968) Chronic studies with sodium saccharin and sodium cyclamate in dogs (Abstract no. 15). *Toxicol. appl. Pharmacol., 12*, 291

Fitzhugh, O.G., Nelson, A.A. & Frawley, J.P. (1951) A comparison of the chronic toxicities of synthetic sweetening agents. *J. Am. pharm. Assoc., 40*, 583-586

Fritz, H. & Hess, R. (1968) Prenatal development in the rat following the administration of cyclamate, saccharin and sucrose. *Experientia, 24*, 1140-1141

Fujita, H., Kobayasi, T., Asagami, C., Iwao, E., Oda, Y. & Mori, T. (1965) Five cases which showed diffuse erythema and edematous papules, possibly caused by saccharin (Jpn.). *Acta dermatol. (Kyoto), 60*, 303-308

Furuya, T., Kawamata, K., Kaneko, T., Uchida, O., Horiuchi, S. & Ikeda, Y. (1975) Long-term toxicity study of sodium cyclamate and saccharin sodium in rats (Abstract). *Jpn. J. Pharmacol., 25*, 55P-56P

Goldstein, R.S., Hook, J.B. & Bond, J.T. (1978) Renal tubular transport of saccharin.

Gordon, H.H. (1972) Allergic reactions to saccharin. *Am. J. Obstet. Gynecol., 113*, 1145

Grasselli, J.G., ed. (1973) *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds,* Cleveland, OH, Chemical Rubber Co., p. B-969

Hartvig, R., Gyllenhaal, O. & Hammarlund, M. (1978) Determination of saccharin in urine by electron-capture gas chromatography after extractive methylation. *J. Chromatogr., 151,* 232-236

Hawley, G., ed. (1977) *The Condensed Chemical Dictionary*, 9th ed., New York, Van Nostrand-Rheinhold, p. 869

Hicks, R.M., Chowaniec, J. & Wakefield, J.StJ. (1978) *Experimental induction of bladder tumors by a two-stage system.* In: Slaga, T.J., Sivak, A. & Boutwell, R.K., eds, *Carcinogenesis*, Vol. 2, *Mechanisms of Tumor Promotion and Cocarcinogenesis,* New York, Raven Press, pp. 475-489

Homburger, F. (1978) *Negative lifetime carcinogen studies in rats and mice fed 50,000 ppm saccharin.* In: Galli, C.L., Paoletti, R. & Vettorazzi, G., eds, *Chemical Toxicology of Food*, Amsterdam, Elsevier/North-Holland Biomedical Press, pp. 359-373

Hooson, J., Hicks, R.M., Grasso, P. & Chowaniec, J. (1980) *ortho*-Toluene sulphonamide and saccharin in the promotion of bladder cancer in the rat. *Br. J. Cancer* (in press)

Hussein, M.M., Jacin, H. & Rodriguez, F.B. (1976) Quantitative determination of saccharin in food products by ultraviolet spectrometry. *J. agric. Food Chem., 24,* 36-40

IARC (1979) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity,* No. 8, Lyon, pp. 90, 183, 300, 460

Ishidate, M., Jr & Odashima, S. (1977) Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* - a screening for chemical carcinogens. *Mutat. Res., 48,* 337-354

Jacin, H, (1975) The quantitative determination of *ortho*-toluene sulfonamide (*o*-TS) in saccharin by U.V. spectrophotometry and by gas chromatography. *Dtsch. Lebensmittel-Rundschau, 71,* 428-429

Jagannath, D.R. & Brusick, D. (1978) *Mutagenicity Evaluation of* ortho-*Toluenesulphoneamide in the Ames* Salmonella/*Microsome Plate Test,* Final Report, submitted to AKZO, Arnhem, The Netherlands by Litton Bionetics Inc., Kensington, MD

Janiak, R.A., Damon, H.H. & Mohr, T.C. (1978) Determination of orthotoluenesulfonamide (OTS) in soluble saccharin. *J. agric. Food Chem., 26*, 710-711

Kakemi, K., Sezaki, H., Muranishi, S. & Tsujimura, Y. (1969) Absorption and excretion of drugs. XL. Enhancement of the rectal absorption of pharmaceutical amines with lauryl sulfate and saccharinate anions. *Chem. pharm. Bull., 17*, 1641-1650

Kennedy, G.L., Jr, Fancher, O.E. & Calandra, J.C. (1976) Subacute toxicity studies with sodium saccharin and two hydrolytic derivatives. *Toxicology, 6*, 133-138

Kline, J., Stein, Z.A., Susser, M. & Warburton, D. (1978) Spontaneous abortion and the use of sugar substitutes (saccharin). *Am. J. Obstet. Gynecol., 130*, 708-711

Klotzsche, C. (1969) The teratogenic and embryotoxic effect of cyclamate, saccharin and sucrose (Ger.). *Arzneimittel-Forsch., 19*, 925-928

Kramers, P.G.N. (1977) Mutagenicity of saccharin in *Drosophila*: the possible role of contaminants. *Mutat. Res., 56*, 163-167

Kristoffersson, U. (1972) The effect of cyclamate and saccharin on the chromosomes of a Chinese hamster cell line. *Hereditas, 70*, 271-282

Kroes, R., Peters, P.W.J., Berkvens, J.M., Verschuuren, H.G., De Vries, T. & van Esch, G.J. (1977) Long term toxicity and reproduction study (including a teratogenicity study) with cyclamate, saccharin and cyclohexylamine. *Toxicology, 8*, 285-300

Lawson, T.A. (1978) *Replication and permeability in the animal model.* In: Guggenheim, B., ed., *Health and Sugar Substitutes*, Basel, Karger, pp. 48-53

Lederer, J. (1977) Saccharin, its impurities and their teratogenic effect (Fr.). *Louvain Med., 96*, 495-501

Lederer, J. & Pottier-Arnould, A.M. (1969) Toxicity of sodium cyclamate and saccharin for the offspring of pregnant mice (Fr.). *Diabète, 17*, 103-106

Lederer, J. & Pottier-Arnould, A.M. (1973) Influence of saccharin on the development of the embryo in the gestating rat (Fr.). *Diabète, 21*, 13-16

Lehmann, K.B. (1929) Feeding study with and without saccharin on mice pairs and search for the minimal toxic effect (Ger.). *Arch. Hyg., 101*, 39-47

Leonard, A. & Leonard, E.D. (1979) Mutagenicity test with saccharin in the male mouse. *J. environ. Pahtol. Toxicol., 2*, 1047-1053

Lessel, B. (1971) *Carcinogenic and teratogenic aspects of saccharin.* In: *SOS/70 Proceedings of the Third International Congress of Food Science and Technology, Washington DC, 1970,* Chicago, IL,Institute of Food Technologists, pp. 764-770

Lethco, E.J. & Wallace, W.C. (1975) The metabolism of saccharin in animals. *Toxicology, 3,* 287-300

Lorke, D. (1969) Studies on the embryotoxic and teratogenic effects of cyclamate and saccharin in the mouse (Ger.). *Arzneimittel-Forsch., 19,* 920-922

Lorke, D. & Machemer, L. (1975) The effect of several weeks' treatment of male and female mice with saccharin, cyclamate or cyclohexylamine sulphate on fertility and dominant lethal effects (Ger.). *Humangenetik, 26,* 199-205

Luckhaus, G. & Machemer, L. (1978) Histological examination of perinatal eye development in the rat after ingestion of sodium cyclamate and sodium saccharin during pregnancy. *Food Cosmet. Toxicol., 16,* 7-11

Lutz, W.K. & Schlatter, C. (1977) Saccharin does not bind to DNA of liver or bladder in the rat. *Chem.-biol. Interactions, 19,* 253-257

Lygre, D.G. (1974) The inhibition by saccharin and cyclamate of phosphotransferase and phosphohydrolase activities of glucose-6-phosphatase. *Biochim. biophys. Acta, 341,* 291-297

Lygre, D.G. (1976) Inhibition by saccharin of glucose-6-phosphatase: effects of alloxan *in vivo* and deoxycholate *in vitro. Can. J. Biochem., 54,* 587-590

Machemer, L. & Lorke, D. (1973) Dominant lethal test in the mouse for mutagenic effects of saccharine. *Humangenetik, 19,* 193-198

Machemer, L. & Lorke, D. (1975) Method for testing mutagenic effects of chemicals on spermatogonia of the Chinese hamster. Results obtained with cyclophosphamide, saccharin, and cyclamate. *Arzneimittel-Forsch., 25,* 1889-1896

Masubuchi, M., Nawai, S., Hiraga, K. & Hirokado, M. (1977) Lack of the cytogenetic effects of saccharin and its impurities on CHO-K1 cells (Jpn.). *Ann. Rep. Tokyo Metr. Res. Lab. P.H., 28,* 159-161

Masubuchi, M., Yoshida, S., Hiraga, K. & Nawai, S. (1978a) The mutagenicity of sodium saccharin (S-Na). II. Cytogenetic studies (Abstract no. 18). *Mutat. Res., 54,* 219

Masubuchi, M., Takahashi, A., Takahashi, O., Yoshida, S., Ando, H., Kudo, K. & Hiraga, K. (1978b) The mutagenicity of sodium saccharin (S-Na). I. Dominant-lethal test (Abstract no. 17). *Mutat. Res., 54*, 218-219

Masubuchi, M., Nawai, S., Hirokado, M. & Hiraga, K. (1978c) Lack of the cytogenetic effects of saccharin impurities on CHO-K1 cells (Abstract no. 13). *Mutat. Res., 54*, 242-243

Matthews, H.B., Fields, M. & Fishbein, L. (1973) Saccharin: distribution and excretion of a limited dose in the rat. *J. agric. Food Chem., 21*, 916-919

McCann, J. (1977) *Short-term tests (Appendix II).* In: *Cancer Testing Technology and Saccharin*, Washington DC, Office of Technology Assessment, Congress of the United States, pp. 91-108

McChesney, E.W., Coulston, F. & Benitz, K.-F. (1977) Six-year study of saccharin in rhesus monkeys (Abstract no. 79). *Toxicol. appl. Pharmacol., 41*, 164

Miller, R., White, L.W. & Schwartz, H.J. (1974) A case of episodic urticaria due to saccharin ingestion. *J. Allerg. clin. Immunol., 53*, 240-242

Minegishi, K.-I., Asahina, M. & Yamaha, T. (1972) The metabolism of saccharin and the related compounds in rats and guinea pigs. *Chem. pharm. Bull., 20*, 1351-1356

Mockute, D. & Bernotiene, G. (1976) Determination of organic additives and their reaction products in electrolytes during metal electroplating. 5. Determination of benzamide, *o*-toluylamide, *o*-toluenesulfonamide, saccharin, phthalimidine, and phthalimide in nickel plating electrolytes when all are present (Russ.). *Liet. TSR Mokslu Akad. Darb., Ser. B, 3*, 87-90 [*Chem. Abstr., 86,* 65144v]

Mohr, U., Green, U., Althoff, J. & Schneider, P. (1978) *Syncarcinogenic action of saccharin and sodium-cyclamate in the induction of bladder tumours in MNU-pretreated rats.* In: Guggenheim, B., ed., *Health and Sugar Substitutes*, Basel, Karger, pp. 64-69

Mondal, S., Brankow, D.W. & Heidelberger, C. (1978) Enhancement of oncogenesis in C3H/10T½ mouse embryo cell cultures by saccharin. *Science, 201*, 1141-1142

Monsanto Industrial Chemicals Co. (undated) *Santicizer®9, Solid Processing Aid and Reactive Plasticizer*, Technical Bulletin IC/PL-9, Newport Beach, CA

Munro, I.C., Moodie, C.A., Krewski, D. & Grice, H.C. (1975) A carcinogenicity study of commercial saccharin in the rat. *Toxicol. appl. Pharmacol., 32*, 513-526

National Formulary Board (1970) *National Formulary XIII*, 13th ed., Washington DC, American Pharmaceutical Association, pp. 120-121, 644-645

National Formulary Board (1975) *National Formulary XIV*, 14th ed., Washington DC, American Pharmaceutical Association, pp. 642-645

National Institute of Hygienic Sciences (1973) *Chronic Toxicity Study of Sodium Saccharin: 21 Months Feeding in Mice*, Tokyo, Japan

National Research Council (1972) *Food Chemicals Codex*, 2nd ed., Washington DC, National Academy of Sciences, pp. 706-707, 764-765

National Research Council (1974) *Food Chemicals Codex*, 2nd ed., 1st Supplement, Washington DC, National Academy of Sciences, pp. 49, 56-58

National Research Council/National Academy of Sciences (1978) *Saccharin: Technical Assessment of Risks and Benefits*, Report No. 1, Committee for a Study on Saccharin and Food Safety Policy, Washington DC, Assembly of Life Sciences/Institute of Medicine

Neumann, R.O. (1926) Is the utilization of food protein influenced by saccharin? (Ger.) *Arch. Hyg., 96*, 265-276

Ochi, H. & Tonomura, A. (1978) Presence of unscheduled DNA synthesis in cultured human cells after treatment with sodium saccharin (Abstract no. 26). *Mutat. Res., 54*, 224

Oser, B.L., Carson, S., Cox, G.E., Vogin, E.E. & Sternberg, S.S. (1975) Chronic toxicity study of cyclamate:saccharin (10:1) in rats. *Toxicology, 4*, 315-330

Pitkin, R.M., Reynolds, W.A., Filer, L.J., Jr & Kling, T.G. (1971a) Placental transmission and fetal distribution of saccharin. *Am. J. Obstet. Gynecol., 111*, 280-286

Pitkin, R.M., Andersen, D.W., Reynolds, W.A. & Filer, L.J., Jr (1971b) Saccharin metabolism in *Macaca mulatta*. *Proc. Soc. exp. Biol. (N.Y.), 137*, 803-806

Poncelet, F., Roberfroid, M., Mercier, M. & Lederer, J. (1979) Absence of mutagenic activity in *Salmonella typhimurium* of some impurities found in saccharin. *Food Cosmet. Toxicol., 17*, 229-231

Pool, B. (1978) Non-mutagenicity of saccharin. *Toxicology, 11*, 95-97

Price, J.M., Biava, C.G., Oser, B.L., Vogin, E.E., Steinfeld, J. & Ley H.L. (1970) Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and sodium. *Science, 167*, 1131-1132

Prőls, H., Haslbeck, M. & Mehnert, H. (1973) Studies on the effect of high doses of saccharin on the metabolism of diabetics (Ger.). *Dtsch. med. Wschr., 98*, 1901-1904

Rao, M.S., & Qureshi, A.B. (1972) Induction of dominant lethals in mice by sodium saccharin. *Indian J. med. Res., 60*, 599-603

Ratchik, E.M., & Viswanathan, V. (1975) GLC determination of saccharin in pharmaceutical products. *J. pharm. Sci., 64*, 133-135

Remsen, I. & Fahlberg, C. (1879) On the oxidation of substitution products of aromatic hydrocarbons. IV. On the oxidation of orthotoluenesulphamide. *J. Am. chem. Soc., 1*, 426-438

Renwick, A.G. (1978) The fate of saccharin impurities: the metabolism and excretion of 3-amino[3-^{14}C]benz[*d*] isothiazole-1,1-dioxide and 5-chlorosaccharin in the rat. *Xenobiotica, 8*, 487-494

Renwick, A.G. & Williams, R.T. (1978) The fate of saccharin impurities: the excretion and metabolism of [3-^{14}C]benz [*d*]isothiazoline-1,1-dioxide (BIT) in man and rat. *Xenobiotica, 8*, 475-486

Renwick, A.G., Ball, L.M., Corina, D.L. & Williams, R.T. (1978) The fate of saccharin impurities: the excretion and metabolism of toluene-2-sulphonamide in man and rat. *Xenobiotica, 8*, 461-474

Riggin, R.M., Kinzer, G.W., Margard, W.L., Mondron, P.J., Girod, F.T. & Birts, M.A. (1978) *Identification, Development of Methods for Analysis and Mutagenicity Testing of Impurities in Sodium Saccharin*, Columbus, OH, Battelle Columbus Laboratories

Roe, F.J.C., Levy, L.S. & Carter, R.L. (1970) Feeding studies on sodium cyclamate, saccharin and sucrose for carcinogenic and tumour-promoting activity. *Food Cosmet. Toxicol., 8*, 135-145

Sabri, M.I., Sharma, S.K. & Krishna Murti, C.R. (1969) Growth inhibitory action of saccharin and cyclamate on rats receiving a poor rice diet. *Br. J. Nutr., 23*, 505-509

Salaman, M.H. & Roe, F.J.C. (1956) Further tests for tumour-inititating activity: *N,N*-di-(2-chloroethyl)-*p*-aminophenylbutyric acid (CB1348) as an initiator of skin tumour formation in the mouse. *Br. J. Cancer, 10*, 363-378

Samuel, B.C. & Rao, M.S. (1972) Induction in *D. melanogaster* with *o*-sulphobenzoic imide (saccharin). *Drosophila Inform. Serv., 48*, 47

Schmähl, D. (1973) Lack of carcinogenic effect of cyclamate, cyclohexylamine and saccharin in rats (Ger.). *Arzneimittel-Forsch., 23*, 1466-1470

Schmähl, D. (1978) Experiments on the carcinogenic effect of ortho-toluol-sulfonamid (OTS). *Z. Krebsforsch., 91*, 19-22

The Sherwin-Williams Company (1977a) *Sodium Saccharin - Non-Nutritive Sweetener*, Technical Data Sheet 1, Chemicals Division, Cleveland, OH

The Sherwin-Williams Company (1977b) *Products Directory*, Technical Bulletin 741, Chemicals Division, Cleveland, OH, p. 3

The Sherwin-Williams Company (1978a) *Saccharin Insoluble Powder FCC - Non-Nutritive Sweetener*, Technical Data Sheet 2, Chemicals Division, Cleveland, OH

The Sherwin-Williams Company (1978b) *Sodium Saccharin Pelletized - Non-Nutritive Sweetener*, Technical Data Sheet 3, Chemicals Division, Cleveland OH

Sieber, S.M. & Adamson, R.H. (1978) *Long-term studies on the potential carcinogenicity of artificial sweeteners in non-human primates*. In: Guggenheim, B., ed., *Health and Sugar Substitutes*, Basel, Karger, pp. 266-271

Simko, J.P., Jr (1977) *Analysis of toothpastes*. In: Senzel, A.J., ed., *Newburger's Manual of Cosmetic Analysis*, 2nd ed., Washington DC, Association of Official Analytical Chemists, pp. 141-146

Smyly, D.A., Woodward, B.B. & Conrad, E.C. (1976) Determination of saccharin, sodium benzoate, and caffeine in beverages by reverse phase high-pressure liquid chromatography. *J. Assoc. off. anal. Chem., 59*, 14-19

Šrám, R.J. & Weidenhofferová, H. (1969) Mutagenic activity of saccharin. *Drosophila Inform. Serv., 44*, 120

Šrám, R.J., & Zudová, Z. (1972) Mutagenic activity of saccharin (Abstract no. 19), *EMS Newsl., 6*, 25

Šram, R.J. & Zudová, Z. (1974) Mutagenicity studies of saccharin in mice. *Bull. environ. Contam. Toxicol., 12*, 186-192

Stavrić, B., Lacombe, R., Watson, J.R. & Munro, I.C. (1974) Isolation, identification, and quantitation of *o*-toluenesulfonamide, a major impurity in commercial saccharins. *J. Assoc. off. anal. Chem., 57*, 678-681

Stavrić, B., Klassen, R. & By, A.W. (1976) Impurities in commercial saccharin. I. Impurities soluble in organic solvents. *J. Assoc. off. anal. Chem., 59*, 1051-1058

Stoltz, D.R., Stavrić, B., Klassen, R., Bendall, R.D. & Craig, J. (1977) The mutagenicity of saccharin impurities. I. Detection of mutagenic activity. *J. environ. Pathol. Toxicol., 1*, 139-146

Stone, D., Matalka, E. & Pulaski, B. (1971) Do artificial sweeteners ingested in pregnancy affect the offspring? *Nature, 231*, 53

Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K. & Gori, G.B. (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. *Cancer Res., 33*, 3069-3085

Strouthes, A. (1978) Mortality and the concurrent ingestion of food and saccharin in rats. *Physiol. Psychol., 6*, 89-92

Szinai, S.S. & Roy, T.A. (1976) Pyrolysis GLC identification of food and drug ingredients. I. Saccharin. *J. chromatogr. Sci., 14*, 327-330

Tanaka, A., Nose, N., Suzuki, T., Kobayashi, S. & Watanabe, A. (1977) Determination of saccharin in soft drinks by a spectrophotometric method. *Analyst, 102*, 367-370

Tanaka, R. (1964) LD_{50} of the saccharin or cyclamate for mice embryo in the 7th day of pregnancy (fetal median lethal dose: FLD_{50}). *J. Iwate med. Assoc., 16*, 330-337

Tanaka, S., Kawashima, K., Nakaura, S., Nagao, S., Kuwamura, T. & Omori, Y. (1973) Studies on the teratogenicity of food additives. I. Effects of saccharin sodium on the development of rats and mice. *J. Food Hyg. Soc., 14*, 371-379

Taylor, J.D., Richards, R.K., Wiegand, R.G. & Weinberg, M.S. (1968) Toxicological studies with sodium cyclamate and saccharin. *Food Cosmet. Toxicol., 6*, 313-327

Taylor, J.M. & Friedman, L. (1974) Combined chronic feeding and three-generation reproduction study of sodium saccharin in the rat (Abstract no. 200). *Toxicol appl. Pharmacol., 29*, 154

Tenenbaum, M. & Martin, G.E. (1977) High pressure liquid chromatographic determination of saccharin in alcoholic products. *J. Assoc. off. anal. Chem., 60*, 1321-1323

Tisdel, M.O., Nees, P.O., Harris, D.L. & Derse, P.H. (1974) *Long-term feeding of saccharin in rats.* In: Inglett, G.E., ed, *Symposium: Sweeteners,* Westport, CN, Avi Publishing Co., pp. 145-158

Ulland, B., Weisburger, E.K. & Weisburger, J.H. (1973) Chronic toxicity and carcinogenicity of industrial chemicals and pesticides (Abstract no. 19). *Toxicol. appl. Pharmacol., 25*, 446

US Department of Commerce (1978) *US Imports for Consumption and General Imports*, FT 246/December 1977, Bureau of the Census, Washington DC, US Government Printing Office, p. 1211

US Department of Health, Education, & Welfare (1973a) *Subacute and Chronic Toxicity, and Carcinogenicity of Sodium Saccharin*, Final Report, Project P-169-170, Division of Pathology, Washington DC, Food & Drug Administration

US Department of Health, Education, & Welfare (1973b) *Histopathologic Evaluation of Tissues from Rats following Continuous Dietary Intake of Sodium Saccharin and Calcium Cyclamate for a Maximum Period of Two Years*, Project P-169-170, Division of Pathology, Washington DC, Food & Drug Administration

US Food & Drug Administration (1977a) Food additives permitted in food for human consumption or in contact with food on an interim basis pending additional study. *Fed. Regist., 42*, 1461-1462

US Food & Drug Administration (1977b) Food additives: saccharin and its salts. *Fed. Regist., 42,* 1486-1487

US Food & Drug Administration (1977c) Saccharin and its salts: proposed rule making. *Fed. Regist., 42*, 19996-20010

US Food & Drug Administration (1978) Saccharin notice. *US Code Fed. Regul.,* Title 21, parts 101.11, 175.105, 180.37

US International Trade Commission (1976a) *Synthetic Organic Chemicals, US Production and Sales, 1974*, USITC Publication 776, Washington DC, US Government Printing Office, p. 120

US International Trade Commission (1976b) *Imports of Benzenoid Chemicals and Products, 1974*, USITC Publication 762, Washington DC, US Government Printing Office, p. 98

US International Trade Commission (1977a) *Saccharin from Japan and the Republic of Korea*, USITC Publication 846, Washington DC, US Government Printing Office, pp. A-4, A-7, A-42 - A-45

US International Trade Commission (1977b) *Synthetic Organic Chemicals, US Production and Sales, 1975*, USITC Publication 804, Washington DC, US Government Printing Office, p. 42

US International Trade Commission (1978a) *Synthetic Organic Chemicals, US Production and Sales, 1977*, USITC Publication 920, Washington DC, US Government Printing Office, pp. 200, 266

US International Trade Commission (1978b) *Imports of Benzenoid Chemicals and Products, 1977*, USITC Publication 900, Washington DC, US Government Printing Office, p. 94

US Pharmacopeial Convention, Inc. (1975) *The US Pharmacopeia*, 19th rev., Rockville, MD, pp. 568-569

US Tariff Commission (1922) *Census of Dyes and other Synthetic Organic Chemicals, 1921,* Tariff Information Series No. 26, Washington DC, US Government Printing Office, p. 25

US Tariff Commission (1940) *Synthetic Organic Chemicals, US Production and Sales, 1939,* Report No. 140, Second Series, Washington DC, US Government Printing Office, p. 48

US Tariff Commission (1954) *Synthetic Organic Chemicals, US Production and Sales, 1953,* Report No. 194, Second Series, Washington DC, US Government Printing Office, p. 114

US Tariff Commission (1974) *Imports of Benzenoid Chemicals and Products, 1973*, TC Publication 688, Washington DC, US Government Printing Office, p. 94

Vesely, D.L. & Levey, G.S. (1978) Saccharin inhibits guanylate cyclase activity: possible relationship to carcinogenesis. *Biochem. biophys. Res. Commun., 81*, 1384-1389

Wade, A., ed.(1977) *Martindale, The Extra Pharmacopoeia*, 27th ed., London, The Pharmaceutical Press, pp. 612-613

Weast, R.C., ed. (1977) *CRC Handbook of Chemistry and Physics*, 58th ed., Cleveland, OH, Chemical Rubber Co., pp. C-529, C-610, C-685

van Went-de Vries, G.F. & Kragten, M.C.T. (1975) Saccharin: lack of chromosome-damaging activity in Chinese hamsters *in vivo*. *Food Cosmet. Toxicol., 13*, 177-183

WHO (1976) Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents, and certain food additives. *WHO Food Addit. Ser., No. 7*, pp. 203-205, 211-216

WHO (1978) Evaluation of certain food additives. Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO tech. Rep. Ser., No. 617*, p. 25

Wild, D., Eckhardt, K., Gocke, E. & Kling, M.-T (1980) *Comparative results of short-term* in vitro *and* in vivo *mutagenicity tests obtained with selected environmental chemicals.* In: Norpoth, K. & Garner, R.C., eds, *Short-Term Mutagenicity Test Systems for Detecting Carcinogens,* Berlin, Springer (in press)

Windholz, M., ed (1976) *The Merck Index,* 9th ed., Rahway, NJ, Merck & Co., p. 1077

Wolff, S. & Rodin, B. (1978) Saccharin-induced sister chromatid exchanges in Chinese hamster and human cells. *Science, 200,* 543-545

Yamada, J. (1975) Artificial sweeteners. *Jpn chem. Rev.,* p. 68

Yoshida, S.M., Masubuchi, M. & Hiraga, K. (1978) Induced chromosome aberrations by artificial sweeteners in CHO-K1 cells (Abstract no. 45). *Mutat. Res., 54,* 262

STUDIES IN HUMANS OF CANCER IN RELATION TO THE CONSUMPTION OF ARTIFICIAL, NON-NUTRITIVE SWEETENING AGENTS

A general discussion of the use of epidemiological studies for establishing carcinogenicity is presented in the preamble to this volume, p. 16. The epidemiological data relating to cyclamates are not adequately separated from those relating to saccharin, and persons taking artificial sweeteners often do not know whether they are taking one or the other, or indeed a mixture of both. This review does not, therefore, consider them separately; however, when a distinction is made in a particular study, this is mentioned.

Case reports

Bladder cancer has been described in four persons who took artificial sweeteners; one took saccharin (4 tablets a day for many years)(Grasset, 1974), and three took cyclamates (Barkin *et al.*, 1977). The latter persons consumed large daily doses of cyclamates, namely 75, 65 and 40-50 mg/kg bw, respectively [A dose of 50 mg/kg bw per day is equivalent to an intake by a 70 kg person of about seventy 50 mg tablets each day]. Two of these patients had diabetes mellitus and two were smokers.

Trends in bladder cancer (Table 1A)[1]

Burbank & Fraumeni (1970) examined bladder cancer death rates in the US for the years 1950-1967. There was no clear-cut break in the continuity of the trends in the age-specific or age-adjusted rates following the widespread introduction of artificial sweeteners (mainly a 10:1 mixture of cyclamate:saccharin) in 1962. There was also no break in the continuity of incidence trends for bladder cancer in Connecticut and New York states.

Armstrong & Doll (1974) carried out a cohort analysis of bladder cancer mortality in England and Wales for the period 1911-1970. This showed 'no evidence of any break in the continuity of the trends in either men or women which corresponds to the introduction of saccharin'.

[While these studies indicate that no marked increase in incidence of bladder cancer has occurred in the US and the UK following the increased use of artificial sweeteners, studies of

[1] The Working Group was aware of a study in progress in which information was being collected on patients with urinary bladder cancer,in particular on occupational exposure, geographical factors and eating habits (e.g., consumption of saccharin and cyclamates) (IARC, 1979).

incidence or mortality trends in populations are likely to be insensitive for three reasons: (1) the proportion of the population exposed to large amounts of artificial sweeteners is small. Therefore, unless there was an excess risk for humans of much higher magnitude than that suggested by experimental work in animals, only a small proportion of bladder cancers in the general population would be attributable to this exposure; (2) changes over time in the exposure of the general population to other risk factors, such as smoking and occupation, would also affect the rates; (3) early diagnosis or improvements in medical treatment or both, which have led to increased survival among patients with bladder cancer, mean that an increase in incidence may not be reflected in an increase in mortality].

Studies of patients with diabetes mellitus (Table 1B)

Kessler (1970) reported on the mortality experience of 21,447 diabetic patients registered at one diabetes clinic in Boston from 1930-1956, who were followed up until the end of 1959. Expected deaths were computed on the basis of the mortality of the population of Massachusetts. Bladder cancer mortality was less than expected in both sexes [for males: observed to expected deaths (O/E) = 14/18.09, Standardized Mortality Ratio (SMR) = 0.77, $P > 0.3$; for females: O/E = 7/11.52, SMR = 0.61, $P > 0.1$]. Respiratory cancer deaths were significantly lower in males (O/E = 46/71.7, SMR = 0.64, $P < 0.005$), a finding which may be only partly explained by the reduced cigarette consumption among diabetic men compared to men in the general population, since males also had lower than expected mortality for cancers other than those of the respiratory tract (O/E = 312/348.78, SMR = 0.89, $P < 0.05$). Females did not show a similar effect for either respiratory cancer (O/E = 25/19.7, SMR = 1.27, $P > 0.2$) or other cancers (O/E = 519/509.81, SMR = 1.01, $P > 0.5$) [There were some differences between the study and control populations (other than the presence of diabetes) which might have affected the results of the study. For instance, 17% of the diabetic group were Jews, compared with only 5% of the Massachusetts population. The study did not measure artificial sweetener consumption in diabetics and did not consider the possible role of sweeteners in the etiology of cancer].

Armstrong & Doll (1975) performed a case-control study using death certificates from England and Wales for the period 1966-1972: 18,733 persons with bladder cancer as the underlying cause of death constituted the cases, and a random sample of 19,709 persons with other cancers as the cause of death were used as controls. Among cases, diabetes mellitus was mentioned on the death certificates of 138 men and 81 women; the corresponding figures in controls (excluding cancer of the lung and pancreas) were 103 and 172. The relative risk of bladder cancer for male and female diabetics combined was 0.98, with 95% confidence limits of 0.70-1.38. Among a sample of 269 patients who died in 1971-1972 and who were chosen to determine the date of diagnosis from medical records, there was no increase in risk of bladder cancer in those who had had diabetes of long duration. An indication of saccharin consumption among diabetics was ascertained from a sample of 200 diabetics currently attending a diabetes clinic in Oxford and 200 controls matched by age and sex currently on the lists of the same general practices as the diabetics. These diabetics were shown to consume substantially more saccharin than the nondiabetic controls, and the duration of regular saccharin use by diabetics was highly correlated with the duration of the diabetes.

Armstrong *et al.* (1976) reported on the mortality experience of 5971 diabetics who were mainly new members of the British Diabetic Association from November 1965 until the end of 1968 and who were followed for 5-8 years to mid-1973. Expected deaths from bladder cancer and SMRs in the cohort of diabetics were calculated by comparison with the mortality experience of the population of England and Wales of comparable age and sex. Deaths from bladder cancer were fewer than expected: O/E = 4/5.8, SMR = 0.70 (not significant). Smoking-related cancers (i.e., those of buccal cavity and pharynx, oesophagus, respiratory system and bladder) were also less frequent - O/E = 30/61.1, SMR = 0.49, $P < 0.01$ - as was non-smoking related cancer - O/E = 98/107.0, SMR = 0.92 (not significant). Deaths from all cancers (128 observed) were significantly fewer than expected (168) (SMR = 0.76, $P < 0.01$). Data on saccharin consumption was gathered from a questionnaire sent to 4000 members of the British Diabetic Association (about 10% of the total membership) and returned by 77%: more than half of them used saccharin tablets daily, with an overall daily intake of 3-6 tablets, depending on age and sex; older men consumed more. Information relating to a sample of 61 survivors from the mortality study (100 were sent questionnaires) indicated that by the end of follow-up 10% (6) would have taken saccharin daily for 25 years or more, a further 13% (8) for between 10 and 25 years and 77% (47) for less than 10 years or not at all.

[The risk of bladder cancer in diabetics who do not use artificial sweeteners may be lower than that in the general population, either because of metabolic differences or differences in, say, their diet, use of drugs, exposure to tobacco or occupational factors. Therefore, derivation of an expectation from the general population for the risk of bladder cancer in diabetic populations may conceal a risk. The studies of diabetics cannot therefore be regarded as providing strong evidence for a lack of a carcinogenic effect of artificial sweeteners in humans. These studies of diabetic patients are the only ones that have investigated the possible carcinogenic effect on organs other than the bladder, and, so far, there is no evidence for such an effect].

Case-control studies

Table 2 summarizes in chronological sequence of publication the 7 case-control studies of bladder cancer available to the Working Group in which subjects were asked how much artificial sweetener they used. These studies will be considered in turn.

Morgan & Jain (1974) reported a study in which histologically confirmed cases of transitional-cell carcinoma of the urinary bladder were individually matched to a control patient according to age and sex. Male controls had benign prostatic hypertrophy, while female controls had stress incontinence. Rates of response to a mailed questionnaire were:

TABLE 1. Epidemiological studies relating to use of artificial sweeteners and bladder cancer

A. Studies of trends in bladder cancer

Reference	Data	Results
Burbank & Fraumeni (1970)	US bladder cancer deaths, 1950-1967 US total cyclamate-saccharin consumption, 1950-1969	No clear-cut increase since widespread introduction of artificial sweeteners
Armstrong & Doll (1974)	UK bladder cancer deaths, 1911-1970 UK *per caput* saccharin consumption, 1939-1972	No evidence of break in trends corresponding to increased saccharin use

B. Studies of patients with diabetes mellitus

Reference	Years	Populations	Relative risk (RR) & standardized mortality ratio (SMR) of bladder cancer	Significance (95% confidence interval)
Kessler (1970)	1930-1956 (registration) 1931-1959 (follow-up)	*Observed:* 14 male and 7 female bladder cancer deaths among 21,447 diabetics at outpatient clinic *Expected:* from age/sex specific rates in Massachusetts over the same period	(m) SMR = 0.8 (f) SMR = 0.6	$P > 0.3$ $P > 0.1$

Table 1 (contd)

Reference	Years	Populations	Relative risk (RR) & standardized mortality ratio (SMR) of bladder cancer	Significance (95% confidence interval)
Armstrong & Doll (1975)	1966-1972	*Observed:* 138 males and 81 females with diabetes mentioned on death certificate out of 18,773 persons who died of bladder cancer in the UK *Expected*: proportion with mention of diabetes in pooled observations of bladder cancer (18,733) and other cancer deaths (not lung or pancreas) (19,709)	(m) RR = 1.00 (f) RR = 0.97	(0.6 - 1.6) (0.6 - 1.6)
Armstrong *et al.* (1976)	1965-1968 (registration) 1965-1973 (follow-up)	*Observed*: 4 bladder cancer deaths among 1207 total deaths in 5971 UK diabetics followed prospectively for 5-8 years *Expected*: from 10% random sample of all deaths in UK, 1972, for similar age/sex	SMR = 0.7	not significant (0.19 - 1.79)

TABLE 2. Summary of case-control studies of bladder cancer in relation to the use of artificial sweeteners

Reference	Years subjects recruited	No. of subjects		Source of information on artificial sweetener consumption
		Cases	Controls	
Morgan & Jain (1974)	Not stated	(m) 158 (f) 74	158 74	MQ
Simon *et al.* (1975)	1965-1971	(f) 135	390	MQ
Howe *et al.* (1977)	1974-1976	(m) 480 (f) 152	480 152	PI
Wynder & Goldsmith (1977)	1969-1974	(m) 132 (f) 31	124 29	PI
Miller *et al.* (1978)	Not stated	(m) 188 (f) 77	376 154	SQ
Connolly *et al.* (1978)	Not stated	(m) 243 (f) 98	479 194	NK
Kessler & Clark (1978)	1972-1975	(m) 365 (f) 154	365 154	PI

m - male
f - female
MQ - mailed questionnaire
PI - personal interview
SQ - supervised questionnaire
NK - not known
NS - not statistically significant

Table 2 (contd)

Source of subjects	Relative risk (95% confidence interval)	Matching variables
Not stated Controls had benign prostatic hypertrophy (m) and stress incontinence (f)	1.00[a] (0.6-1.8)[f] 0.35[a] (0.1-0.8)[f]	Sex, age (+ 5 yrs)
White subjects from 10 hospitals Controls without urinary problems from same hospitals	Cyclamate 1.2[b] (0.5-2.6) Saccharin 1.0[b] (0.5-1.7)	Age (± 4 yrs), urban/rural, discharge date
All incident bladder cancer cases in 3 provinces in Canada Neighbourhood controls	1.6[c] (1.1-2.3) 1.6[c] (0.3-1.1)[f]	Sex, age (± 5 yrs)
Bladder cancer patients from 17 hospitals Controls without 'tobacco-related disease'	0.7[c] (0.2-2.2) 0.7[c] (0.1-15)	Sex, race, hospital status, age (± 5 yrs)
All patients over 40 years at urology clinic: cases, bladder cancer; controls, all others	1.1[b] (NS) 0.9[b] (NS)	Sex, age (± 5 yrs)
Not stated	0.9[d] (0.6-1.4)[f] 0.7[d] (0.4-1.3)[f]	Sex, age (± 5 yrs), residence
All bladder cancer patients in 19 hospitals Controls in same hospital cancer-free, no bladder complaints	1.1[e] (0.8-1.6) 0.8[e] (0.5-1.4)	Sex, race, age (± 3 yrs), marital status

[a] Prolonged regular use *versus* never use

[b] Usual adult use *versus* never use

[c] Ever use *versus* never use

[d] Extent of use not specified

[e] Use for 6 months or more *versus* never use; relative risk adjusted for many variables, including smoking, occupation, diabetes

[f] Confidence intervals not given in the published paper but calculated by the Working Group (approximate intervals for the ratio of discordant pairs in matched studies, or calculated using the method of Miettinen, 1969, 1970)

male cases, 67% and male controls, 57%; female cases, 73% and female controls, 57%. The actual analysis was based on 158 matched male case-control pairs and 74 matched female pairs. Data relating to 2 cases (1%) and 18 controls (10%) in men, and 17 cases (18%) and 17 controls (18%) in women could not be analysed due to lack of a suitable match. Prolonged regular use of any artificial sweetener was associated with a relative risk of 1.00 (not significant) in males and 0.35 ($P < 0.01$) in females [The distribution of amount, frequency and duration of artificial sweetener consumption were not given, and the method of ascertaining the cases was not specified. It is not clear how many of the nonascertained subjects and those who were sent questionnaires had already died and to what extent the study was therefore one of long-term survivors of bladder cancer. The controls had diseases the treatment of which may have affected fluid intake, although this was not medically prescribed, and thus their intake of artificial sweeteners may also have been affected. For example, control women may have drunk less to reduce stress incontinence. On the other hand, women with stress incontinence might be more likely to be obese than cases and thus have a higher consumption of artificial sweeteners].

From pathology records and the diagnostic indices of 10 hospitals in Massachusetts (other than Boston) and Rhode Island, Simon *et al.* (1975) identified 216 white women in whom lower urinary tract cancer (95% of which were bladder cancer) was diagnosed between 1965 and 1971. Three female controls without urinary tract problems were matched to each case according to race, age (± 5 years), place of residence (urban/rural) and hospital. Forty of the cases were found to have died and 77% of the remainder responded to a mail questionnaire, leaving 135 cases for analysis. The corresponding response rate among controls was 72%. Neither saccharin (RR, 1.0) nor cyclamate (RR, 1.2) were significantly associated with lower urinary tract cancer. The absence of such an effect was demonstrated in tea- as well as coffee-drinkers [No data were presented on the possible risk of cancer in relation to amount, frequency or duration of artificial sweetener use].

A study by Howe *et al.* (1977) is the only one that reported a positive association between artificial sweetener use and bladder cancer. Cases were derived from all nonrecurrent, newly-diagnosed bladder cancer patients in 3 Canadian provinces between April 1974 and June 1976. They were identified through population-based tumour registries, pathologists and urologists. Out of 821 eligible cases, 56 were dead, 65 refused to be interviewed, 25 were too ill and 34 were not approached because their doctors did not want their patients to be interviewed, thus leaving 641. The final analysis was actually based on 632 cases (480 men and 152 women), representing 77% of all the eligible cases. The cases were interviewed in their homes, and controls were sought by approaching neighbours who lived a specified number of homes away from the case and continuing from one home to another until a control of the same sex and age (within 5 years) was found. A statistically significant excess of artificial sweetener use was reported in male cases (RR, 1.6); and this excess was also present when the analysis was restricted to the 82% of men who used artificial sweeteners that contained only saccharin. The relative risks of bladder cancer in male users of these brands (relative to those in men who had never consumed saccharin) were 1.5 and 2.1 among men who consumed less than 2500 and 2500 or more saccharin tablets per year, respectively.

Similarly, the relative risks were 1.4 and 2.0 among men who used such saccharin tablets for less than 3 years and more than 3 years, respectively. The trends in both associations were statistically significant (P=0.02, P=0.03). The risk of bladder cancer was lower among female users of artificial sweeteners than among women who never used them (RR, 0.6; 95% confidence interval, 0.3-1.1). The possible confounding effects of smoking and coffee consumption on the risk of bladder cancer were analysed after discarding pairs discordant with respect to smoking or coffee consumption, using the following groupings: ⩽10,000 and >10,000 packs of cigarettes lifetime consumption and 'never' and 'ever' instant coffee consumption [This analysis for confounding was described as inadequate in a *Lancet* editorial article (Anon., 1977), and the study was thus considered to be inconclusive].

In response to the *Lancet* editorial, Miller & Howe (1977) presented further analyses relating to men to better control for smoking and coffee consumption. This analysis was unmatched. When instant coffee consumption was considered in three groups (none, <1½ cups per day and ⩾1½ cups per day), the relative risk for each group and the summary relative risk remained unchanged, at 1.6 [95% confidence interval, 1.1-2.4]. The authors also divided cigarette consumption into 5 groups, namely: none (RR, 0.7), former smokers of <5000 packs lifetime consumption (1.5), former smokers of ⩾5000 packs lifetime consumption (2.1), current smokers of <15 cigarettes/day (1.0) and current smokers of ⩾15 cigarettes/day (1.7). The summary relative risk was given as 1.7 (P=0.01, one-tailed); but this excluded data relating to nonsmokers [Inclusion of the latter lowered the summary relative risk estimate to 1.5 (P=0.03, one-tailed; 95% confidence interval, 1.0-2.3].

Howe *et al.* (1979) have also analysed their data on men using a logistic regression analysis which took account of the case-control matching. Cigarette consumption was related to both artificial sweetener use and bladder cancer and was the principal confounding variable. When the confounding variables (life-time tobacco consumption, 'high risk' occupations, use of non-public water supply, bladder infection, diabetes, school marks, lifetime aspirin use, daily coffee use) were controlled for simultaneously, the relative risk of bladder cancer in relation to artificial sweetener consumption (divided into 5 groups) was as follows:

Artificial sweetener consumption (tablets/day)	Relative risk (95% confidence interval)
0	1.0
1-4	0.9 (0.4-2.1)
5-6	1.6 (0.6-4.3)
7-8	1.1 (0.3-4.0)
9 or more	2.8 (0.9-8.9)

The authors reported that when artificial sweetener use was considered as a continuous variable, the overall linear trend is statistically significant using a one-tailed test (P=0.03) [This implies that the trend is not likely to be statistically significant at the conventional level of P=0.05 if a two-tailed test were used].

When the analysis was restricted to those individuals who reported using saccharin alone, the results were similar. The relative risk estimates for up to 4, 4-8, and more than 8 tablets of saccharin per day were: 0.9, 1.4 and 3.1 (Howe *et al.*, 1979) [The Working Group noted that the confidence limits were not reported].

[The numbers of men in each artificial sweetener consumption group are not given in the paper; however, the Working Group inferred, on the basis of the width of the reported confidence intervals (see table above), that they were small. While the data are consistent with no effect of artificial sweetener use on the risk of bladder cancer, there remains a suspicion of such an effect among heavy users of artificial sweeteners. An unknown number of eligible neighbourhood controls who were not at home when the interviewer visited could not be included in the study. It is possible that factors (e.g., social) influencing their absence were also related to saccharin consumption and so may have biassed the estimates of relative risk].

Wynder & Goldsmith (1977) studied bladder cancer patients and controls matched for sex, race, age and 'hospital status'. Thirteen of 132 male cases (10%) and 16 of 124 male controls (13%) used artificial sweeteners (RR, 0.7; not significant). The comparable data for females were 4 of 31 cases (13%) and 5 of 29 controls (17%) (RR, 0.7; not significant) [It was not clearly stated whether each case-control pair came from the same hospital; the subjects were recruited from 17 hospitals throughout the USA].

Miller *et al.* (1978) studied 265 patients with bladder cancer and 530 matched controls (two for each case) matched for sex and age (± 5 years). All subjects were registered as out-patients at a Canadian urology clinic. Data were collected using a self-administered questionnaire supervised by clinic staff [The diagnosis was unknown to both patient and staff at the time the data were collected]. There was no significant risk associated with the regular use of artificial sweeteners (RR, 1.1 for men and 0.9 for women) [The diagnoses of controls are not given, and no account was taken of possible confounding factors, such as smoking].

Connolly *et al.* (1978) published a letter reporting no excess of artificial sweetener users in 341 patients with bladder cancer, compared with 673 controls matched for sex, age (± 5 years) and place of residence (county and urban/rural) [The relative risk was 0.93 for men and 0.70 for women. No information was provided on how the cases and controls were ascertained or on the distribution or effect of potential confounding variables. No data were presented on the extent, duration or length of exposure to artificial sweeteners].

Kessler & Clark (1978), expanding the work of Kessler (1976), ascertained all of 1300 histologically confirmed bladder cancer cases discharged from 19 Baltimore hospitals between 1972 and 1975. Of these, 519 (40%) (365 males, 154 females) were interviewed; the remainder consisted of subjects who died (509), those who were unable or refused to be interviewed (115) and those who were identified late (157). One control patient without a diagnosis of cancer and without a bladder condition was matched with each case on the basis of hospital, age (± 3 years), sex, race, date of admission and current marital status. Personal interviews were used to obtain information on the use of foods and beverages containing artificial sweeteners by frequency, quantity, duration and brand name. Use of saccharin or cyclamates during the year prior to the cancer diagnosis was ignored for each case and matched control. Matched-pair analysis of relative risks for those using any form of artificial sweeteners 'more than occasionally' were 0.97 for men (95% confidence limits, 0.70-1.35), 1.00 (0.63-1.59) for women and 0.98 (0.75-1.28) overall. Adjustment of these figures for such potential confounding factors as smoking, occupation, obesity and diabetes yielded relative risks of 1.11 (0.78-1.58) for men, 0.80 (0.47-1.39) for women and 1.04 (0.80-1.40) overall in users of 6 months' duration or more. No evidence of a dose-response trend was obtained for either sex when users were subdivided into three equal groups according to lifetime exposure. Relative risks calculated separately for saccharin were 1.08 (0.79-1.48) for men and 0.87 (0.55-1.37) for women; for cyclamates these figures were 1.12 (0.79-1.58) and 0.74 (0.46-1.19) [These results make a relative risk of about 1.5 or higher unlikely, but they are not inconsistent with a relative risk closer to 1. Restriction of the cases to survivors leaves open the possibility that use of artificial sweeteners might be higher in cases with rapidly lethal tumours, who would have been less likely to be interviewed].

[Neither Howe *et al.* (1977) nor Kessler & Clark (1978) found a statistically significant difference in the proportion of cases and controls who ate foods or drank beverages containing artificial sweeteners. However, intake of sweeteners from these sources may be of too short duration and involve too young an age group to allow detection of an effect].

General

Examination of time trends in the USA and in England and Wales shows that there has been no marked increase in the incidence of bladder cancer following the rapid increase in use of artificial sweeteners. In the UK, diabetics as a group consume higher quantities of artificial sweeteners than the general population and experience a lower mortality from bladder cancer than the general population. However, because of metabolic differences or differences in diet, use of drugs, exposure to tobacco or occupational factors in diabetics, this finding cannot exclude a carcinogenic effect of sweeteners.

Seven case-control studies were considered by the Working Group. Five were negative but were limited by some inadequacies in experimental design. Only two examined possible confounding factors in detail. Of these, one suggested that artificial sweetener use was positively associated with bladder cancer in men but not in women. The association was limited to men who used nine or more tablets of artificial sweeteners per day or if only saccharin was considered, who consumed an average of more than eight tablets of saccharin per day; the relative risk in both instances was about 3. However, in these small groups, the result could have been due to chance, to confounding factors that were not included in the analysis, or (as in any study with relative risks near 1) to residual effects of those confounding factors that were considered in the analysis.

In 6 out of the 7 case-control studies reviewed, women with bladder cancer took less artificial sweeteners than the controls, and in one study this difference was statistically significant. This observation provides no evidence that artificial sweeteners cause bladder cancer in women.

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.

Footnote

After the meeting of the Working Group, two epidemiological investigations (Morrison & Buring, 1980; Wynder & Stellman, 1980) were reported.

The study by Morrison & Buring evaluated the relation between cancer of the lower urinary tract and the use of artificial sweeteners in a case-control study of 592 patients with lower-urinary-tract cancer (94 per cent of whom had a bladder tumour) and 536 controls chosen from the general population of the study area. A history of use of artificial sweeteners and exposure to other known or suspected risk factors was determined by interview. In those who had used dietetic beverages and in those who had used sugar substitutes, the relative risk of lower-urinary-tract cancer was estimated as 0.9 (0.7 to 1.2, 95% confidence interval), as compared with 1 in nonusers of artificial sweeteners. Among men, the relative risk was 0.8 (0.6 to 1.1) in those who had used dietetic beverages and 0.8 (0.5 to 1.1) in those who had used sugar substitutes. Among women, the corresponding relative risks were 1.6 (0.9 to 2.7) and 1.5 (0.9 to 2.6). Increasing frequency or duration of use of artificial sweeteners was not consistently associated with increasing relative risk. This study suggests that, as a group, users of artificial sweeteners have little or no excess risk of cancer of the lower urinary tract [Authors' summary].

The study by Wynder & Stellman was a case-control study of 302 men and 65 women with bladder cancer and an equal number of controls matched for age, sex, hospital and hospital-room status. No association was found between the use of artificial sweeteners or diet-beverage consumption and bladder cancer. The relative risk of bladder cancer (95% confidence interval) among men was 0.9 (0.7-1.3) for artificial sweetener use and 0.8 (0.6-1.2) for diet-beverage consumption; among women, the relative risks were 0.6 (0.3-1.4) and 0.6 (0.3-1.3), respectively. These relative risk estimates did not vary appreciably when a number of potential confounding variables were controlled for, namely, history of diabetes, obesity, occupation, education, religion and coffee or tea consumption. No dose-response relationships between consumption of artificial sweeteners or diet beverages and quantity or duration of use were observed.

References

Anon. (1977) Bladder cancer and saccharin. *Lancet, ii*, 592-593

Armstrong, B. & Doll, R. (1974) Bladder cancer mortality in England and Wales in relation to cigarette smoking and saccharin consumption. *Br. J. prev. soc. Med., 28*, 233-240

Armstrong, B. & Doll, R. (1975) Bladder cancer mortality in diabetics in relation to saccharin consumption and smoking habits. *Br. J. prev. soc. Med., 29*, 73-81

Armstrong B., Lea, A.J., Adelstein, A.M., Donovan, J.W., White, G.C. & Ruttle, S. (1976) Cancer mortality and saccharin consumption in diabetics. *Br. J. prev. soc. Med., 30*, 151-157

Barkin, M., Comisarow, R.H., Taranger, L.A. & Canada, A. (1977) Three cases of human bladder cancer following high dose cyclamate ingestion. *J. Urol., 118*, 258-259

Burbank, F. & Fraumeni, J.F., Jr (1970) Synthetic sweetener consumption and bladder cancer trends in the United States. *Nature, 227*, 296-297

Connolly, J.G., Rider, W.D., Rosenbaum, L. & Chapman, J.-A. (1978) Relation between the use of artificial sweeteners and bladder cancer. *Can. med. Assoc. J., 119*, 408

Grasset, A.V. (1974) Saccharin and carcinoma of the bladder. *Can. med. Assoc. J., 110*, 1135,1137

Howe, G.R., Burch, J.D., Miller, A.B., Morrison, B., Gordon, P., Weldon, L., Chambers, L.W., Fodor, G. & Winsor, G.M. (1977) Artificial sweeteners and human bladder cancer. *Lancet, ii*, 578-581

Howe, G.R., Burch, J.D., Miller, A.B., Cook, G.M., Esteve, J., Morrison, B., Gordon, P., Chambers, L.W., Fodor, G. & Winsor, G.M. (1979) Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *J. natl Cancer Inst.* (in press)

IARC (1979) *Directory of On-Going Research in Cancer Epidemiology, 1979 (IARC Scientific Publications No. 28)*, Lyon, Abstract no. 203, p. 108

Kessler, I.I. (1970) Cancer mortality among diabetics. *J. natl Cancer Inst., 44*, 673-686

Kessler, I.I. (1976) Non-nutritive sweeteners and human bladder cancer: preliminary findings. *J. Urol., 115*, 143-146

Kessler, I.I. & Clark, J.P. (1978) Saccharin, cyclamate, and human bladder cancer. No evidence of an association. *J. Am. med. Assoc., 240*, 349-355

Miettinen, O.S. (1969) Individual matching with multiple controls in the case of all-or-none response. *Biometrics, 25*, 339-355

Miettinen, O.S. (1970) Estimation of relative risk from individually matched series. *Biometrics, 26*, 75-86

Miller, A.B. & Howe, G.R. (1977) Artificial sweeteners and bladder cancer. *Lancet, ii*, 1221-1222

Miller, C.T., Neutel, C.I., Nair, R.C., Marrett, L.D., Last, J.M. & Collins, W.E. (1978) Relative importance of risk factors in bladder carcinogenesis. *J. chron. Dis., 31*, 51-56

Morgan, R.W. & Jain, M.G. (1974) Bladder cancer: smoking, beverages and artificial sweeteners. *Can. med. Assoc. J., 111*, 1067-1070

Morrison, A.S. & Buring, J.E. (1980) Artificial sweeteners and cancer of the lower urinary tract. *New Engl. J. Med., 302,* 537-541

Simon, D., Yen, S. & Cole, P. (1975) Coffee drinking and cancer of the lower urinary tract. *J. natl Cancer Inst., 54*, 587-591

Wynder, E.L. & Goldsmith, R. (1977) The epidemiology of bladder cancer. A second look. *Cancer, 40*, 1246-1268

Wynder, E.L. & Stellman, S.D. (1980) Artificial sweetener use and bladder cancer: a case control study. *Science* (in press)

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

[4] Gerard, S.S., Gardner, B., Patti, J., Husain, V., Shouten, J. & Alfonso, A.E. (1980) Effects of triiodothyronine and reserpine on induction and growth of mammary tumors in rats by 3-methylcholanthrene. *J. surg. Oncol.*, *14*, 213-218

[5] Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M. & Sugiyama, T. (1980) *Cooperative programme on short-term assays for carcinogenicity in Japan.* In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Molecular and Cellular Aspects of Carcinogen Screening Tests* (*IARC Scientific Publications No. 27*), Lyon, pp. 323-330

[6] Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K. & Neal, S.B. (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. *Environ. Mutagenesis*, *3*, 11-21

SACCHARIN (Group 3)

A. Evidence for carcinogenicity to humans (*inadequate*)

There is no consistent evidence that the risk of cancer is increased among users of saccharin[1]. Three case-control studies have shown no overall excess of bladder cancer in association with use of artificial sweeteners, nearly all of which contained saccharin[2-4]. The largest of these studies was a population-based survey in 10 areas of the US, involving interviews with 3010 bladder cancer patients and 5783 controls randomly selected from the areas in which the patients resided[2]. The relative risk of bladder cancer associated with any use of artificial sweeteners was 0.99 (95% confidence limits, 0.89-1.10) among men and 1.07 (0.89-1.29) among women, compared with a risk of 1.0 among non-users. However, significant trends of increasing risk with increasing average daily consumption and with duration of use were observed in certain subgroups, namely female non-smokers (with a low baseline risk of bladder cancer) and male heavy smokers (with a high baseline risk). Since these subgroups were considered *a priori* to be worthy of special attention on the basis of hypotheses derived from animal experimentation, the findings raise the possibility that saccharin may act as a weak carcinogen and/or promoter. In one of the other two studies, a population-based survey of 592 patients with lower-urinary-tract cancer[3], the relative risk among women associated with any use of diet drinks or sugar substitutes was 1.6 and exceeded by two-fold that for non-smokers. The risk for any use among men was 0.8.

B. Evidence for carcinogenicity to animals (*limited*)

Saccharin or sodium saccharin has been tested, either alone or in combination with other chemicals, by several routes of administration. (a) It was tested by oral administration of several dose levels to different strains of mice and rats, including several multigeneration studies[1]. In one study in mice and in one in rats the tumour incidence was similar in treated and control animals. In one single-generation study and two two-generation studies in rats, a significant increase in the incidence of bladder tumours was

observed in males treated with high doses[1,5]. Several experiments in mice, rats, hamsters and monkeys were considered inadequate for evaluation. A 10:1 mixture of sodium cyclamate:sodium saccharin was given in one multigeneration experiment in mice and in two experiments in rats[1]. Transitional-cell carcinomas of the bladder were induced only in male rats of one strain given the highest dose. Pretreatment with a single instillation into the bladder of a low dose of *N*-nitroso-*N*-methylurea or feeding of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and subsequent oral administration of saccharin increased the incidence of bladder neoplasms in female and male rats, respectively[1]. Commercial saccharin preparations enhanced lung tumour induction in mice when given before or during urethane administration[5]. (b) Saccharin and sodium saccharin were also tested by insertion into the urinary bladder of mice, inducing bladder neoplasms[1]. (c) Experiments in which it was given by skin application or intraperitoneal administration could not be evaluated[1].

ortho-Toluenesulphonamide increased the incidence of bladder neoplasms in one out of three experiments in rats following its oral administration[1].

C. Evidence for activity in short-term tests (*inadequate*)

Saccharin was not mutagenic[1,6-8] and did not induce DNA repair in bacteria[9]. It produced genetic effects in yeast[10]; it was not mutagenic to *Drosophila melanogaster*[6]; it was reported to be mutagenic in mouse lymphoma cells in the presence of a liver homogenate[1]. Conflicting results were obtained with regard to chromosomal anomalies in cells *in vitro*[1,11]: saccharin caused chromosomal aberrations in cultured Chinese hamster cells[1] and a low level of sister chromatid exchanges in cultured lymphocytes and hamster cells[1,12]. No anomaly was seen in cells from animals treated *in vivo*[1,6]. It showed no covalent binding to DNA of rat bladder or liver[1]. It did not produce transformation in mouse embryo fibroblasts ($C3H/10T_{\frac{1}{2}}$)[11], but at high concentrations it enhanced the transformation of these cells by 3-methylcholanthrene[1]. No sperm abnormality was seen in mice[13]. There were conflicting data concerning the production of dominant lethal mutations in mice[1], and conflicting results in the specific-locus somatic mutation test (spot-test) conducted in mice *in vivo*: in one study[14], positive but non-dose related effects were obtained; while negative results were obtained in single-dose experiments reported in another study[15]. No data on humans were available.

	DNA damage	Mutation	Chromosomal anomalies	Other
Prokaryotes	-	-		
Fungi/Green plants		+		
Insects		-		
Mammalian cells *(in vitro)*		?	?	T(-)
Mammals *(in vivo)*	-	?	-	DL(?) SA(-)
Humans *(in vivo)*				

T = cell transformation; DL = dominant lethal mutations; SA = sperm abnormalities

References

[1] *IARC Monographs, 22*, 111-186, 1980

[2] Hoover, R.N. & Strasser, P.H. (1980) Artificial sweeteners and human bladder cancer. Preliminary results. *Lancet, i*, 837-840

[3] Morrison, A.S. & Buring, J.E. (1980) Artificial sweeteners and cancer of the lower urinary tract. *New Engl. J. Med., 302*, 537-541

[4] Wynder, E.L. & Stellman, S.D. (1980) Artificial sweetener use and bladder cancer: A case-control study. *Science, 207*, 1214-1216

[5] Theiss, J.C., Arnold, L.J. & Shimkin, M.B. (1980) Effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice. *Cancer Res., 40*, 4322-4324

[6] Eckhardt, K., King, M.-T., Gocke, E. & Wild, D. (1980) Mutagenicity study of Remsen-Fahlberg saccharin and contaminants. *Toxicol. Lett., 7*, 51-60

[7] Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M. & Sugiyama, T. (1980) *Cooperative programme on short-term assays for carcinogenicity in Japan.* In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Molecular and Cellular Aspects of Carcinogen Screening Tests* (*IARC Scientific Publications No. 27*), Lyon, pp. 323-330

[8] De Flora, S. (1981) Study of 106 organic and inorganic compounds in the *Salmonella*/microsome test. *Carcinogenesis, 2*, 283-298

[9] Rosenkranz, H.S. & Leifer, Z. (1980) *Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient* Escherichia coli. In: de Serres, F.J. & Hollaender, A., eds, *Chemical Mutagens. Principles and Methods for their Detection*, Vol. 6, New York, Plenum Press, pp. 109-147

[10] Moore, C.W. & Schmick, A. (1979) Recombinogenicity and mutagenicity of saccharin in *Saccharomyces cerevisiae. Mutat. Res., 67*, 215-219

[11] Saxholm, H.J.K., Iversen, O.H., Reith, A. & Brøgger, A. (1978) Carcinogenesis testing of saccharin. No transformation or increased sister chromatid exchange observed in two mammalian cell systems. *Eur. J. Cancer, 15*, 509-513

[12] Renner, H.W. (1979) Possible mutagenic activity of saccharin. *Experientia, 35*, 1364-1365

[13] Topham, J.C. (1980) Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat. Res., 74*, 379-387

[14] Mahon, G.A.T. & Dawson, G.W.P. (1982) Saccharin and the induction of presumed somatic mutations in the mouse. *Mutat. Res., 103*, 49-52

[15] Fahrig, R. (1982) Effects in the mammalian spot test: Cyclamate *versus* saccharin. *Mutat. Res., 103*, 43-47

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

[15]Norseth, T., Andersen, A. & Giltvedt, J. (1983) Cancer incidence in the rubber industry in Norway. *Scand. J. Work Environ. Health, 9 (Suppl. 2)*, 69-71

[16]Wilcosky, T.C., Checkoway, H., Marshall, E.G. & Tyroler, H.A. (1984) Cancer mortality and solvent exposures in the rubber industry. *Am. ind. Hyg. Assoc. J., 45*, 809-811

[17]Wang, H.-W., You, X.-J., Qu, Y.-H., Wang, W.-F., Wang, D., Long, Y.-M. & Ni, J.A. (1984) Investigation of cancer epidemiology and study of carcinogenic agents in the Shanghai rubber industry. *Cancer Res., 44*, 3101-3105

[18]Arp, E.W., Jr, Wolf, P.H. & Checkoway, H. (1983) Lymphocytic leukemia and exposures to benzene and other solvents in the rubber industry. *J. occup. Med., 25*, 598-602

[19]Gustavsson, P., Hogstedt, C. & Holmberg, B. (1986) Mortality and incidence of cancer among Swedish rubber workers. *Scand. J. Work Environ. Health, 12*, 538-544

[20]Veys, C. (1982) *The rubber industry: reflections on health risks.* In: Gardner, A.W., ed., *Current Approaches to Occupational Health*, Vol. 2, Bristol, Wright PSG, pp. 1-29

[21]*IARC Monographs, Suppl. 6*, 488, 1987

SACCHARIN (Group 2B)

A. Evidence for carcinogenicity to humans (*inadequate*)

The evidence that the risk of cancer is increased among users of artificial sweeteners is inconsistent[1]. Since the positive report of Howe *et al.*[2], reports have become available on seven case-control studies and on one population study of bladder cancer.

The largest was a population-based study in ten areas of the USA, with 3010 bladder cancer cases and 5783 controls. The relative risk for bladder cancer associated with use of artificial sweeteners was 1.0 (95% confidence interval, 0.9-1.1) among men and 1.1 (0.9-1.3) among women. Significant trends of increasing risk with increasing average daily consumption were found in certain subgroups examined *a priori* on the basis of the results of animal experiments; these subgroups were female nonsmokers and male heavy smokers[3]. Subsequent, independent re-analysis 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[4]. In response, the original investigators noted that the inconsistency derived from the development of risk scores which, in their opinion, were not correctly derived, as two relevant variables had been omitted[5]. In a subsequent report on data from one of the areas participating in this study, the use of hospital and population controls was compared. A higher proportion of hospital controls was found to have used artificial sweeteners than population controls[6]. This had been postulated earlier[2] as a possible reason for the negative findings of a hospital-based case-control study[7]. Bias resulting from use of prevalent rather than incident cases[8] has been suggested as a possible reason for the negative findings of another hospital-based case-control study[9].

Three other case-control studies have also shown increased risks among subgroups. In one, conducted simultaneously in Japan, the UK and the USA, the relative risks among women in the US component of the study associated with 'any' use of diet drinks and of sugar substitutes were 1.6 and 1.5, respectively, and 2.6 and 2.1, respectively, for nonsmokers[10]. In the other two areas, however, a history of the use of sugar substitutes, primarily saccharin, was not associated with an elevated bladder cancer risk[11]. In a second study, conducted in West Yorkshire, UK, elevated risks were found for saccharin takers who were nonsmokers. In men, the relative risk was 2.2 (95% confidence interval, 1.3-3.8); that in women was 1.6 (0.8-3.2)[12]. In a third study, conducted in a rural district of Denmark, a relative risk of 2.5 (1.0-6.6) was reported for saccharin consumption in men and women combined. This risk was not reduced after controlling for tobacco use and industrial work[13].

Two studies in Denmark[14,15], one in the USA[16] and a further case-control study in Canada[17], however, gave negative results. In one of the Danish studies, incidence of bladder cancer at ages 20-34 among people born 1941-1945 (when use of saccharin was high in Denmark) was compared with that among those born 1931-1940. The risk for men was 1.0 (0.7-1.6) and that for women, 0.3 (0.1-1.0). This study indirectly assessed intrauterine exposure to saccharin[14]. The other two studies were population-based case-control studies of bladder cancer. In Denmark, the relative risk for people of each sex combined was 0.8 (0.6-1.1)[15]. In a study in the USA of bladder cancer in women aged 20-49, the odds ratio for regular use of artificially sweetened beverages, table-top sweetener or both was 1.1 (0.7-1.7)[16]. In Canada, the odds ratio for use of saccharin was 1.0 (0.9-1.2) in men and 1.0 (0.8-1.2) in women[17]. The increased risks seen in subgroups in other studies were not replicated in either study.

In the USA, in a study of 1862 patients hospitalized for cancer and of 10 874 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[18].

B. Evidence for carcinogenicity to animals (*sufficient*)

Saccharin (unspecified or commercial) has been tested for carcinogenicity by oral administration to mice, rats and hamsters. In mice, saccharin produced no difference in tumour incidence between treated and control animals in one single- and in one multi-generation study. Two further studies by oral administration in mice and three in rats were considered to be inadequate for evaluation. A study in hamsters by oral administration and one study in mice by skin application could not be evaluated. A study in mice by bladder insertion provided evidence for the induction of bladder carcinomas[1]. Oral administration to mice produced thyroid tumours[19].

Sodium saccharin has been tested for carcinogenicity by oral administration to mice, rats and monkeys. One study in mice was inadequate for evaluation[1]. One single-generation study in rats showed an increased incidence of bladder tumours in males; two further studies showed a few bladder tumours; another study showed no difference in tumour incidence between treated and control animals; and two others were inadequate for evaluation[1]. In four two-generation studies in rats, sodium saccharin produced a statistically significant

increase in the incidence of bladder tumours in F_1 males fed either 5% or 7.5% sodium saccharin[1,20]. In a further two-generation study of rats, a dose-related increase in the incidences of benign, malignant and/or combined bladder neoplasms was observed in males treated with doses ranging from 4-7.5% in the diet, while no tumorigenic effect was observed with 1%[21,22]. Transplacental exposure of rats to sodium saccharin and to saccharin (commercial) did not produce any treatment-related neoplasm[21,23]. Sodium saccharin has also been tested in mice by bladder insertion: it increased the incidence of bladder carcinomas. Experiments in which it was tested by oral administration to monkeys and by intraperitoneal administration to mice were considered to be inadequate for evaluation[1].

The combination of sodium saccharin with sodium cyclamate in a ratio of 1:10 has been tested by oral administration in a multigeneration experiment in mice and in single experiments in rats. In one study in rats, transitional-cell carcinomas in the bladder were produced in male animals given the highest dose; in two further studies in rats and in the study in mice, there was no difference in tumour incidence between treated and control animals[1,24]. Another study in rats was inadequate for evaluation[1].

Pretreatment with a single instillation into the 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 bladder neoplasms in rats over that induced by the nitrosourea or the amide alone[1]. Simultaneous administration of *N*-nitroso-*N*-(4-hydroxybutyl)butylamine and sodium saccharin significantly enhanced the induction of bladder papillomas over that seen after treatment with the nitrosamine alone[25]. Commercial saccharin preparations enhanced lung tumour induction in mice when given before or during intraperitoneal administration of urethane[26]. In rats, oral administration of sodium saccharin significantly increased the incidence of bladder neoplasms induced by ulceration of bladder mucosa[27,28]. Other studies of simultaneous or consecutive treatment with saccharin and known carcinogens were inadequate for evaluation[1].

ortho-Toluenesulphonamide was tested for carcinogenicity by oral administration in rats in a two-generation study: no increase in bladder tumour incidence was noted in animals of either generation. In one of two single-generation studies in rats, benign and malignant bladder tumours were found[1].

C. Other relevant data

No data were available on the genetic and related effects of saccharin, sodium saccharin or *ortho*-toluenesulphonamide in humans[29].

It should be noted that many studies do not differentiate between saccharin ('insoluble' form) and sodium saccharin. Additionally, when it is reported that 'saccharin' (presumably sodium saccharin) causes a positive response, primarily in assays for chromosomal effects, the effect is seen only with very high concentrations, at which simple salts also give responses[29].

Treatment of mice with saccharin did not induce micronuclei or chromosomal aberrations in bone-marrow cells or spermatocytes; conflicting results were obtained for the induction of dominant lethal mutations. A commercial preparation (of unknown purity) caused somatic mutations in the mouse spot test. Injection of radioactive saccharin into rats revealed no DNA binding in the liver or bladder, nor did treatment of rats result in DNA damage in bladder tissue. Saccharin did not induce sister chromatid exchanges in cultured human lymphocytes. Negative results were obtained in assays for transformation in cultured rodent cells, but saccharin enhanced transformation of virus-infected rat embryo cells and of C3H 10T1/2 mouse embryo cells initiated with 3-methylcholanthrene in two-state transformation assays. Results obtained with rodent cell systems were inconclusive with regard to inhibition of intercellular communication. It caused DNA strand breaks in rat hepatocytes but no chromosomal aberration in Chinese hamster cells. Saccharin induced aneuploidy but not recombination or gene conversion in yeast. It was not mutagenic and did not induce prophage in bacteria[29].

Treatment of mice with sodium saccharin did not induce micronuclei, somatic mutations (in the spot test) or sperm abnormalities. Treatment of Chinese hamsters did not induce chromosomal aberrations in bone-marrow cells or spermatogonia but induced sister chromatid exchanges in bone-marrow cells. Treatment of mice with commercial sodium saccharin resulted in the induction of dominant lethal mutations, but treatment with a preparation 'purified' by undefined criteria did not. Sodium saccharin induced chromosomal aberrations and sister chromatid exchanges in cultured human lymphocytes and induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells but no mutation in mouse lymphoma cells. It did not induce transformation of BALB/c 3T3 cells. Contradictory results have been reported concerning the ability of sodium saccharin to induce sex-linked recessive lethal mutations in *Drosophila*, and it did not cause a significant increase in heritable translocations. Sodium saccharin induced mutation, gene conversion and recombination in yeast, but was not mutagenic to bacteria[29].

ortho-Toluenesulphonamide did not induce micronuclei or somatic mutation (in the spot test) in mice treated *in vivo*. Contradictory results have been obtained for the induction of sex-linked recessive lethal mutations in *Drosophila*. It was not mutagenic to bacteria[29].

References

[1]*IARC Monographs*, *22*, 55-109, 171-185, 1980

[2]Howe, G.R., Burch, J.D., Miller, A.B., Cook, G.M., Estève, J., Morrison, B., Gordon, P., Chambers, L.W., Fodor, G. & Winsor, G.M. (1980) Tobacco use, occupation, coffee, various nutrients and bladder cancer. *J. natl Cancer Inst.*, *64*, 701-713

[3]Hoover, R.M. & Strasser, P.H. (1980) Artificial sweeteners and human bladder cancer. Preliminary results. *Lancet*, *i*, 837-840

[4]Walker, A.M., Dreyer, N.A., Friedlander, E., Loughlin, J., Rothman, K.J. & Kohn, H.I. (1982) An independent analysis of the National Cancer Institute study on non-nutritive sweeteners and bladder cancer. *Am. J. public. Health*, *72*, 376-381

[5]Hoover, R. & Hartge, P. (1982) Non-nutritive sweeteners and bladder cancer. *Am. J. public Health, 72*, 382-383

[6]Silverman, D.T., Hoover, R.N. & Swanson, G.M. (1983) Artificial sweeteners and lower urinary tract cancer: hospital vs. population controls. *Am. J. Epidemiol., 117*, 326-334

[7]Wynder, E.L. & Stellman, S.D. (1980) Artificial sweetener use and bladder cancer: a case-control study. *Science, 207*, 1214-1216

[8]Goldsmith, D.F. (1982) Calculation of potential bias in the odds ratio illustrated by a study of saccharin use and bladder cancer. *Environ. Res., 27*, 298-306

[9]Kessler, I.I. & Clark, J.P. (1978) Saccharin, cyclamate, and human bladder cancer. No evidence of an association. *J. Am. med. Assoc., 240*, 349-355

[10]Morrison, A.S. & Buring, J.E. (1980) Artificial sweeteners and cancer of the lower urinary tract. *New Engl. J. Med., 302*, 537-541

[11]Morrison, A.S., Verhoek, W.G., Leck, I., Aoki, K., Ohno, Y. & Obata, K. (1982) Artificial sweeteners and bladder cancer in Manchester, UK, and Nagoya, Japan. *Br. J. Cancer, 45*, 332-336

[12]Cartwright, R.A., Adib, R., Glashan, R. & Gray, B.K. (1981) The epidemiology of bladder cancer in West Yorkshire. A preliminary report on non-occupational aetiologies. *Carcinogenesis, 2*, 343-347

[13]Mommsen, S., Aagaard, J. & Sell, A. (1983) An epidemiological study of bladder cancer in a predominantly rural district. *Scand. J. Urol. Nephrol., 17*, 307-312

[14]Jensen, O.M. & Kamby, C. (1982) Intra-uterine exposure to saccharin and risk of bladder cancer in man. *Int. J. Cancer, 29*, 507-509

[15]Møller-Jensen, O., Knudsen, J.B., Sørensen, B.L. & Clemmesen, J. (1983) Artificial sweeteners and absence of bladder cancer risk in Copenhagen. *Int. J. Cancer, 32*, 577-582

[16]Piper, J.M., Matanoski, G.M. & Tonascia, J. (1986) Bladder cancer in young women. *Am. J. Epidemiol., 123*, 1033-1042

[17]Risch, H.A., Burch, J.D., Miller, A.B., Hill, G.B., Steele, R. & Howe, G.R. (1987) Dietary factors and the incidence of cancer of the urinary bladder. *Am. J. Epidemiol.* (in press)

[18]Morrison, A.S. (1979) Use of artificial sweeteners by cancer patients. *J. natl Cancer Inst., 62*, 1397-1399

[19]Prasad, O. & Rai, G. (1986) Induction of papillary adenocarcinoma of thyroid in albino mice by saccharin feeding. *Indian J. exp. Biol., 24*, 197-199

[20]Taylor, J.M., Weinberger, M.A. & Friedman, L. (1980) Chronic toxicity and carcinogenicity to the urinary bladder of sodium saccharin in the in utero-exposed rat. *Toxicol. appl. Pharmacol., 54*, 57-75

[21]Schoenig, G.P., Goldenthal, E.I., Geil, R.G., Frith, C.H., Richter, W.R. & Carlborg, F.W. (1985) Evaluation of the dose response and in utero exposure to saccharin in the rat. *Food chem. Toxicol., 23*, 475-490

[22]Squire, R.A. (1985) Histopathological evaluation of rat urinary bladders from the IRDC two-generation bioassay of sodium saccharin. *Food chem. Toxicol., 23*, 491-497

[23]Schmähl, D. & Habs, M. (1980) Absence of a carcinogenic response to cyclamate and saccharin in Sprague-Dawley rats after transplacental application. *Arzneimittelforschung, 30*, 1905-1906

[24]Schmähl, D. & Habs, M. (1984) Investigations on the carcinogenicity of the artificial sweeteners sodium cyclamate and sodium saccharin in rats in a two-generation experiment. *Arzneimittel-forschung, 34*, 604-606

[25]Nakanishi, K., Hirose, M., Ogiso, T., Hasegawa, R., Arai, M. & Ito, N. (1980) Effects of sodium saccharin and caffeine on the urinary bladder of rats treated with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. *Gann, 71*, 490-500

[26]Theiss, J.C., Arnold, L.J. & Shimkin, M.B. (1980) Effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice. *Cancer Res., 40*, 4322-4324

[27]Cohen, S.M., Murasaki, G., Fukushima, S. & Greenfield, R.E. (1982) Effect of regenerative hyperplasia on the urinary bladder: carcinogenicity of sodium saccharin and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. *Cancer Res., 42*, 65-71

[28]Hasegawa, R., Greenfield, R.E., Murasaki, G., Suzuki, T. & Cohen, S.M. (1985) Initiation of urinary bladder carcinogenesis in rats by freeze ulceration with sodium saccharin promotion. *Cancer Res., 45*, 1469-1473

[29]*IARC Monographs, Suppl. 6*, 488-493, 520-522, 1987

SHALE-OILS (Group 1)

A. Evidence for carcinogenicity to humans (*sufficient*)

The association between shale-oils and skin cancers, particularly of the scrotum, was demonstrated by analyses of 65 cases of skin cancer, including 31 of the scrotum, from the Scottish shale-oil industry. In the UK, over 2000 cases of skin cancer ('mule-spinners' cancer') were recorded among cotton-textile workers and others exposed to lubricating oils (many of which are believed to have been shale-derived). The occupational etiology of these cases is supported by occupational mortality statistics for the UK and by an occupational comparison with fatal cases of penile cancer. In contrast, one study showed very few scrotal cancers among US cotton-textile workers employed in mills where shale-derived lubricants were not used. A cohort study of shale-oil workers in western USA showed statistically significant excesses of all cancers and of colon cancer, although data on duration and time since first exposure were not available. A cohort study of shale-oil workers in Estonia showed a significant excess of skin cancer but not of cancers at other sites[1]. A follow-up of 6064 men who had worked in the Scottish oil-shale industry between 1950 and 1962 showed a significant excess of skin cancer[2]. A case-control study of lung cancer in the shale area showed no association with work in the shale industry[2].

Two basal- and two squamous-cell carcinomas were found among 325 workers employed at an oil-shale demonstration facility during 1948-1969 in Utah, USA. The incidence was about that expected[3].

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.