Carcinogenicity of Saccharin in Laboratory Animals and Humans:

Letter to Dr. Harry Conacher of Health Canada*

Limitations of Dose-Response and Mechanistic Studies

High dietary doses of sodium saccharin cause urinary bladder tumors in rats. Many chemicals are known to cause bladder cancer, even in humans, including certain aromatic amines and azo dyes, as well as cigarettes. Recently, however, the suggestion has been made that saccharin may be different from other bladder carcinogens. It has been argued that a large dose-response study shows that a "threshold" exists between 1% and 3% saccharin in the diets of male rats, and that levels below that are not carcinogenic.1 Hence, the argument goes, saccharin is a non-genotoxic carcinogen that can have a threshold response below which human exposure should not be a health concern. The proponents of that argument theorize that high levels of sodium saccharin increase urinary sodium levels and pH, which lead to the formation of the bladder of precipitates (containing calcium phosphate, silicate, alpha 2u-globulin, saccharin, and other substances), which in turn leads to irritation, hyperplasia, and ultimately tumors.

While those studies may be relevant to evaluating saccharin carcinogenicity, they do not exculpate saccharin as a bladder carcinogen in male rats. The dose-response study was not large enough to evaluate the effects of the lowest tested doses of dietary sodium saccharin in Charles River CD male rats. The authors of the study found that the incidence of bladder tumors in controls was 0/324, compared with 5/658 in the 1% dose group.† The authors state that that difference was not statistically significant. Nevertheless, the lack of significance could have been due to the number of animals tested. The proposal that saccharin is only a non-genotoxic carcinogen is, however, only a theory, which is not supported by available animal and human data. The exact shape of the dose-response curve at low doses of saccharin in one strain and sex of rat, let alone in the genetically diverse human population, is completely unknown.§

† The 4-3 vote is not binding on the director of NIHEH, but carries significant weight in the director’s final decision.
§ Considering that in no case is the precise mechanism of action of any carcinogen known, the statement that a "threshold" has been established in the carcinogenesis tests of saccharin and that this enables regulators to establish a safe level of exposure is unacceptable. In almost every dose-response study of even a potent carcinogen, there is an apparent "threshold" dose, but a regulator who established on that basis an acceptable exposure to, for example, nitrosodi-
Other research also indicates flaws in the theoretical exoneration of saccharin. Some studies have shown that exposure to saccharin does not increase the urinary pH and osmolality. It has been noted that a 7.5% sodium saccharin diet "scarcely represents a large increase from the usual daily dietary intake of sodium ion." Furthermore, saccharin causes bladder cancer not only in male rats but also in female rats, whose urine has lower levels of protein and a higher pH. The mechanism by which bladder tumors develop in females exposed to saccharin has not been well investigated.

Besides critically evaluating the rodent bladder-cancer studies, we urge the Health Protection Branch (HPB) to evaluate carefully several lines of evidence—tumors in rodents at sites other than the bladder, co-carcinogenicity, genotoxicity, epidemiology—that raise additional serious questions about saccharin's safety.

Rodent Chronic-feeding Studies

Several studies on rats and mice found that saccharin causes tumors not just in the urinary bladder, but at additional sites. Other studies show that saccharin promotes tumors initiated by known chemical carcinogens. Some of that research is reviewed below.

Some rodent studies did not find increases in tumor rates following exposures to large doses of sodium saccharin. Some of those studies focused on the urinary bladder without systematic histopathologic examination of other organs, so tumors at sites other than the bladder could have been overlooked. Also, the strains of rodents used varied among studies (for instance, the only studies using Osborne-Mendel rats found tumors at sites other than the bladder). Thus, the absence of reported tumors in some studies may mean only that affected organs were not examined or that the strain was not susceptible. Human variability strongly suggests that any finding of carcinogenesis in any strain of animal should be cause for great concern and caution.

Induction of tumors at sites other than the urinary bladder in rats. In rats:

- The two-generation WARF study found a dose-dependent increased incidence of ovarian plus uterine tumors that did not quite reach statistical significance (controls: 1/17; 0.05%; 1/17; 0.5%; 3/15; 5%; 6/20, p = 0.07). Appendix 1, p. 36
- Bio-research Consultants' one-generation study found small numbers of fore-stomach and skin tumors in treated male rats but not in controls (0%; 0/16; 1%; 2/28; 5%; 3/26).5

Several studies found increased rates of tumors in "all organs." (The lack of monotonic dose-response increases does not necessarily negate the finding of a higher rate in the lower-dosage group.)

- Chowaniec and Hicks found that saccharin increased the incidence of non-bladder tumors in male rats (controls: 1/52; 2 g/kg/day: 11/71; 4 g/kg/day: 7/70).
- Bio-Research Consultants found increased rates of non-bladder tumors in male rats (controls: 3/16; 1%: 15/28; 5%: 7/26).5

The National Academy of Sciences' 1978 review concluded: "An increase in benign uterine tumors and ovarian lesions in saccharin-treated rats was suggested in a few studies." Panel 1 The NAS stated further, "[A]s a result of detailed analyses of the pathology data, the committee concludes that the experimental evidence suggests that ingestion of saccharin at the 5% or 7.5% dietary level may have contributed to an increase in benign uterine tumors and ovarian lesions in female rats." Appendix 1 The NAS's meta-analysis of four studies found a significant increase in uterine tumors (p = 0.041) for the F₀ generation and an increase that almost reached significance (p = 0.063) for the F₀ + F₁ generations.

Induction of tumors at sites other than the urinary bladder in mice. Mice have been less well studied than rats. Positive findings include:

- Bio-research Consultants found:
  1. Saccharin caused an increased incidence of vascular tumors (males: controls: 1/19, 1%; 2/29, 5%; 10/34; females: controls: 1/17, 1%; 5/28, 5%; 7/36). The Congressional Office of Technology Assessment (OTA) said those data support "an association between saccharin and an increase in total and vascular tumors in males; furthermore, the number of vascular tumors was increased in saccharin-fed female mice." Appendix 1
  2. Saccharin caused an increased rate of lung tumors in males (controls: 11%; 1%; 48% (p = 0.007); 5%: 27%). OTA dismissed the higher rate in the 1% group, because an additional increase did not occur at 5% Appendix 1 but the lack of an increase at 5% does not disprove the finding at the lower dosage.
  3. Male mice developed squamous epithelium tumors (skin or fore-stomach) in the 1% group (4/29); no such tumors occurred in the controls or the 5% group.5
  4. Male mice exposed to saccharin had higher incidences of tumors at all sites examined (controls: 4/19; 1%; 20/29, p = 0.002; 5%: 21/34, p = 0.005).5
  5. An analysis by the U.S. National Cancer Institute of this study concluded that "for tumors of the uterus among female mice the life table analysis reveals a significant effect in the high-dose group for females." Appendix 1

- The National Institute of Hygienic Sciences (Tokyo) found a higher incidence of tumors (all sites examined) in female mice at doses of 0.2%, 1%, and 5% saccharin in a 21-month study.5 Incidences of uterine cancer also increased at all three dosages (p = 0.00547 for comparison of controls with all doses com-

methyline or nitrosodiethylamine, would be considered negligible.3

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bined). OTA said, "If the data for uterine cancers at 21 months are considered alone, cancer incidence appears to increase with saccharin dose. However, the data do not convincingly show that saccharin caused or did not cause cancer. . . . Those in the treated animals is higher."

- Thyroid tumors occurred in 5/10 male and 3/10 female mice fed 1.5 g/kg/day saccharin for one year (tumor incidence in controls was not reported).8

**Co-carcinogenicity studies in rats.** In addition to being tested by itself, saccharin has been tested in rodents following their exposure to a known carcinogen. Some of those studies demonstrated that saccharin is a cancer promoter. In rats:

- A co-carcinogenicity study done by the U.S. National Center for Toxicological Research (NCTR) found that 5% acetic acid saccharin in the diets of female rats exposed to N-methyl-N-nitrosourea (MNU) increased the incidence of bladder tumors in dead and moribund animals from 3% to 17%. The researchers considered dead and moribund animals—about 60% of all the animals used—especially important because they reflect both a dose response and an indication of time to tumor. That study indicates that it is not just the sodium salt of saccharin that increases the risk of cancer.

- Another leg of the NCTR co-carcinogenicity study found that dosages of sodium saccharin as low as 0.1% increased tumor rates in dead and moribund animals controls: 2/66; 0.1% saccharin: 7/86; 0.5%: 11/64 (p < 0.018); 1%: 8/46 (p < 0.001); 2.5%: 10/27 (p < 0.002). The 5% group had a tumor rate of only 1/34, presumably reflecting a competing toxicity. There was no apparent threshold for saccharin's effect. For the study as a whole, the tumor rate was increased at 2.5% saccharin. The authors stated, "[S]odium saccharin induced a decrease in time to tumor as observed in dead and moribund animals." They further noted that no changes in urinary composition "were associated with increasing levels of sodium saccharin in the diet. Thus, no threshold dose of saccharin was observed which could be related to gross changes in urine composition." They concluded, "saccharin acts as a tumor promoter in this two-stage carcinogenesis model system by decreasing the latency period of the lesions."

- Male and female rats were given N-butyl-N-(4-hydroxybutyl) nitrosoamine (BBN) for four weeks, followed by various levels of sodium saccharin for 32 weeks.9 There were dose-dependent increases in the numbers of rats with simple hyperplasia or papillary or nodular hyperplasia. In male rats, increases were statistically significant only when saccharin constituted 5% of the diet, but smaller increases were seen at lower levels, including 0.4% saccharin. In females, significant increases in simple, papillary, or nodular hyperplasia were seen at 1% saccharin, with smaller increases at 0.04% and 0.2% saccharin. This study was limited by high levels of hyperplasia in rats exposed only to BBN, by the small numbers of rats in each group (about 30), and by the short duration of the study. The authors stated, "Apparently, doses up to 100 ppm saccharin in the diet are 'no effect' or threshold levels for hyperplasia induction in both sexes, and doses from 2,000 (0.2%) to 50,000 ppm [5%] are potent enhancers."

- Female rats were given varying levels of sodium saccharin for four weeks prior to, overlapping with, or after exposure to MNU. Despite the brief exposure to saccharin, tumor rates were higher (double in some cases) in several test groups, though the differences did not reach statistical significance at the 0.05 level.10

**Co-carcinogenicity studies in mice.** In mice:

- Several studies demonstrated that saccharin, when implanted in bladders in cholesterol pellets, increased by severalfold the incidence of urinary-bladder tumors.11,12 (Saccharin, which is absorbed quickly from the pellet, may have acted as an initiator, the pellet as a promoter.)

As in all chronic-toxicity studies, the association in rodents between saccharin consumption and tumors in the bladder, uterus, ovary, skin, fore-stomach, lungs, and vascular system—with some tumor types occurring in two or more studies or both species—does not prove causality in every case, but the occurrence of such tumors suggests a significant public health issue and that further research is needed.

In addition, saccharin caused bladder cancer when female rats were pretreated with MNU or BBN and when saccharin-cholesterol pellets were implanted in the bladders of mice. Co-carcinogenicity is of particular concern because humans are exposed to a wide variety of toxic agents in their food, water, air, drugs (e.g., tobacco smoke), and workplaces. It is relevant that the International Agency for Research on Cancer (IARC) has found that the urinary bladder is the main target organ for 11 known human carcinogens.14 Obviously, there are many carcinogens whose effects saccharin might promote.

The dosages of saccharin that appear to have elicited effects in some laboratory-animal studies are not much greater than the amounts that some North Americans have consumed. In one study, 0.5%, and possibly 0.1%, dietary saccharin (following exposure to MNU) appeared to increase the incidence of bladder tumors in female rats; another co-carcinogenicity study (using BBN as the initiator) found increased hyperplasia at 1% saccharin and possibly levels as low as 0.04%. Other studies on rats and mice found an increased risk at 1% dietary saccharin (Bio-Research Consultants). If, arguendo, a dose of 0.1% saccharin were considered the no-effect level, that is equivalent to just 50 mg/kg bw/day,15 or
just four times higher than the amount ingested by a 90th-percentile adult consumer in the United States in 1977–1978 and just twice as high as that in the diet of a child in the 90th percentile of consumption. That hardly gives one much confidence that saccharin is safe for human consumption.

Genotoxicity

Several studies have shown that saccharin causes dominant lethal mutations. In mice:

- 1.72% sodium saccharin in the drinking water of male mice (CBA strain) led to a sixfold increase at four weeks in the percentage of intrauterine deaths of offspring of females to which the males had been mated. When injected intraperitoneally in male mice (ICR strain), several different dosing regimens of sodium saccharin led to statistically significant (p < 0.01 and < 0.001) increases in dominant lethal mutations, usually two to four weeks after one or several injections. In a third study, sodium saccharin administered five times intraperitoneally and orally to male mice (ICR strain) caused significant (p < 0.001) decreases in implantations and live fetuses per corpora lutea. A single intraperitoneal dose caused significant (p < 0.01) decreases of the same two measures. Subcutaneous injection of saccharin in two inbred strains of mice caused dominant lethal mutations (p < 0.01). Considering that the dominant-lethal test is rather insensitive, the fact that several studies found that saccharin causes such mutations should be of great concern. Chemicals that cause dominant-lethal mutations are generally found to be carcinogens. Thus, these genotoxicity studies provide strong evidence that supports the other laboratory-animal research indicating that saccharin is a genotoxic cancer initiator.

Epidemiologic Studies

The question of whether saccharin consumption increases the risk of bladder cancer in humans is significant, considering that bladder cancer is the fourth most common cancer in Canadian males (the rate is lower in females). In 1969, the incidence of bladder cancer in males was 23.8 per 100,000. That incidence rose sharply over the following decade and remained at 30 to 32 cases per 100,000 between 1978 and 1988. After that, the incidence declined, to an estimated 23.6 per 100,000 in 1996. It is conceivable that the increase and subsequent decrease were related partly to the increased use of saccharin in the 1960s and early 1970s, followed by the ban on using saccharin in processed foods. Numerous case-control studies have sought to evaluate the relationship between artificial-sweetener consumption (saccharin and cyclamate were generally used together) and the incidence of bladder cancer. Several studies, including some of the largest ones, found significant increases in rates of bladder cancer.

- The National Cancer Institute (3,010 total cases) found relative risks (RRs) for bladder cancer of between 1.5 and 2.0 in several subgroups of Americans, including low-risk white females and heavy-smoking males. Furthermore, with all males and females combined, this study found relative risks of 1.5 to 1.64 among people who consumed two or more diet drinks and six or more servings of artificial sweeteners per day (95% confidence intervals were not given). Sturgeon et al.'s analysis (1,860 cases of the NCI data described above found that heavy use of artificial sweeteners was associated (RR = 2.2) with higher-grade, poorly differentiated bladder tumors. Howe et al. (682 cases) found an increased risk of bladder cancer in Canadian males (RR = 1.6); men who consumed more artificial sweeteners or consumed artificial sweeteners for longer periods of time had relatively high risks.

- Carwright et al. (622 existing cases; 219 new) found an increased risk (RR = 2.2) in British nonsmoking males, but not females. Morrison and Burting (592 male and female patients with lower-urinary-tract cancer—94% of whom had bladder cancer) found increased risks in women who consumed dietetic beverages (RR = 1.8 [1.0–3.3]) and who consumed sugar substitutes (RR = 1.9 [1.0–3.6]) (stratified for age and smoking history). Women who consumed dietetic beverages for five years or more had a relative risk of 3.7.

- Morrison (555 British cases) found an increased risk (RR = 2.3) in British females (but not males or Japanese cases) who consumed more than 10 tablets of sugar substitutes (primarily saccharin) a day.

- A small study (47 female cases) in Denmark found increased risks in all women (RR = 6.7) and in nonsmoking women (RR = 3.3).

Thus, in numerous studies, artificial-sweetener consumption was associated with significant increased risks of bladder cancer in humans, though there were inconsistencies in the risks to men and women. Some (mostly smaller) studies did not find an association. The NTP acknowledges that "a small increased risk in some subgroups, such as heavy users of artificial sweeteners, cannot be unequivocally excluded." (That is an understatement that could have been expressed equally accurately as: Several studies found an increased risk in some subgroups, and it is the subgroup of heavy consumers about whom we should be especially concerned.) That some studies did not detect an increased risk could be real or due to the limited durations of sub-


††Cigarette smoking, a cause of bladder cancer, declined in males by 55% and in females by 32% between 1965 and 1995 (data from the National Clearinghouse on Smoking and Health, Ottawa). Any effect would be likely in a subgroup of heavy users than in light users, all users, or ever users.

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7. Nam J, Gare J. Interim report on statistical analysis of animal testing studies on saccharin. May 29, 1974. (Cited in Reuber)
22. Sturgeon SR, Hargre J, et al. Associations between bladder cancer risk fac-

Appendix

Comments on Acceptable Daily Intake

In 1993, the WHO/FAO's Joint Expert Committee on Food Additives (JECFA) reviewed the safety of saccharin. That committee concluded that saccharin did not pose a cancer risk and established an acceptable daily intake (ADI) of 3 mg/kg body weight (bw)/day. While we disagree with the committee's conclusion about saccharin's carcinogenicity, we recognize that Health Canada or other agencies, following JECFA's lead, might establish an ADI. We believe that JECFA set the ADI at far too high a level, a level that could endanger the public health.

JECFA's ADI was based on a highest-see-observeable-effect level (HNOEL) of 1% saccharin in rodent studies. In fact, however, 1% has not been established as the HNOEL. As noted above, in several studies tumor rates were increased in rodents that consumed 1% saccharin or less.

- Ovarian and uterine tumors at 0.5% saccharin in the two-generation rat study (increase was not statistically significant, but significance might have been reached in a larger study; WARF)

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• Total non-bladder tumors at 1% saccharin in male rats (Bio-Research Consultants).

• Lung, squamous epithelium (skin/fore-stomach), and all-sites tumors at 1% saccharin in male mice (Bio-Research Consultants).

• Urinary bladder tumors in female rats ingesting 0.5% saccharin following one-time exposure to MNU; there was also an increase at 0.1% that did not reach statistical significance, though significance might have been reached in a larger study.

• Increased hyperplasia in the urinary bladder of female rats was caused by 1% dietary saccharin for 32 weeks following exposure to BBN; nonsignificant increases occurred at levels of 0.2% and 0.04% saccharin.

Such data do not support the finding of a threshold for saccharin’s carcinogenic action. However, for the sake of argument, one might consider the HNOEL to be 0.1% saccharin (equivalent to 50 mg/kg bw/day) or 0.05%—tenfold lower than the 0.5% effect level in the 1986 co-carcinogenicity study of West et al.2 (equivalent to 25 mg/kg bw/day). Applying a 100-fold safety factor to those levels would yield an ADI of 0.5 mg/kg bw-day or 0.25 mg/kg bw-day.

In 1977-1978 in the United States, 3-5-year-old children in the 90th percentile of saccharin consumption were consuming 19.67 mg/kg bw-day saccharin.2 U.S. men and women between the ages of 19 and 34 in the 90th percentile were consuming 10.19-10.48 mg/kg bw/day. Those levels of consumption are significantly higher than what JECFA considered acceptable, and far higher than an ADI based on an HNOEL of 0.1% or 0.05% dietary saccharin.

Dr. Robert Maronpot, chief of the laboratory of experimental pathology at the U.S. National Institute of Environmental Health Sciences, provided his comparisons of rat and human exposure, as shown in the Attachment (slide shown at the October 31, 1997, meeting of the National Toxicology Program’s Board of Scientific Counselors). He assumed that the no-observed-effect level (NOEL) for urinary bladder cancer in male rats was 1% dietary saccharin. Using the U.S. Environmental Protection Agency’s default assumption for rodent-to-human comparisons (body weight0.75), he concluded that 1% saccharin in a rat’s diet is only ten times greater than that in the diet of a child in the 90th percentile of consumption (U.S. Department of Agriculture, 1977 survey of consumption) and 13 times greater than that in the diet of an adult in the 90th percentile of consumption. Clearly, if saccharin did cause bladder cancer in rats, it could pose a significant risk to humans who consume large amounts of saccharin.

**Update on Saccharin**

In 1997, the Board of Scientific Counselors, a group of nongovernmental experts convened by the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences, voted 4-3 to continue listing saccharin in the *Report on Carcinogens*. Two previous internal government committees had voted 7-3 and 6-2 to delist saccharin, and one subsequent such committee voted 6-3 to delist. In 2000, the NTP removed saccharin from its listing of carcinogens. Later that year, Congress repealed the law that required a cancer warning notice on foods containing saccharin. Whether the delisting of saccharin will lead to greater consumer exposure remains to be seen, considering saccharin’s bitter aftertaste and the availability of several other artificial sweeteners. Of greater import is whether the considerable weight that the NTP gave to theoretical mechanistic considerations, notwithstanding voluminous experimental data from animal and human studies, portends greater use in the future of that approach for other environmental chemicals.

—EDITOR-IN-CHIEF