



May 18, 1998

Dr. C. W. Jameson  
National Toxicology Program  
Report on Carcinogens  
MD EC-14  
PO Box 12233  
Research Triangle Park, NC 27709

Dear Dr. Jameson:

**RE: NTP's Call for Public Comments; Agents, Substances, Mixtures  
and Exposure Circumstances Proposed for Listing in or  
Removing From the Report on Carcinogens, Ninth Edition --  
Saccharin**

The Calorie Control Council ("the Council") is an international association of manufacturers of low-calorie, reduced-fat and light foods and beverages, including the manufacturers of a variety of sweeteners and other low calorie ingredients used in those products. Manufacturers and users of saccharin are among the Council's members.

In 1996, the Council petitioned the National Toxicology Program (NTP) to delist saccharin from NTP's Report on Carcinogens under its new criteria for delisting which provide for the consideration of data indicating that the agent acts through mechanisms which do not operate in humans and would therefore reasonably be anticipated not to cause cancer in humans.

Saccharin continues to be an important sweetener in food, beverages and over-the-counter products, including dentifrices. The Calorie Control Council submitted the petition with the strong belief that the totality of the saccharin research overwhelmingly supports its safety for human consumption.

After careful review of the October 31, 1997 saccharin deliberations of NTP's Board of Scientific Counselors, the Council submits these comments on: (1) the issues incorrectly presented or presented without adequate scientific perspective or interpretation by the Board and (2) those issues for which NTP specifically requested comments in its March 19, 1998 Call for Public Comments.

## **Epidemiology**

Saccharin has been the subject of numerous epidemiological studies. Evaluation of these studies confirms that there is no detectable association between human bladder cancer and saccharin.

The saccharin epidemiology research has been reviewed numerous times by authoritative bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the International Agency for Research on Cancer (IARC), and the World Cancer Research Fund/American Institute for Cancer Research\* as well as individual experts. All agree that the numerous epidemiological studies on artificial sweeteners (AS) have not substantiated any increase in risk in humans with saccharin use.

There have been no consistent positive results in the many studies reported although isolated subgroups inconsistently have been identified as having a numerically higher relative risk (RR) in a given study. Howe et al. (1977) reported increased risk with use of tabletop sweeteners but not dietetic drinks, for men, but not women. [In a more recent study, the same researchers were unable to substantiate this finding, noting “this study tends to confirm reports of a lack of association between use of artificial sweeteners and subsequent risk of bladder cancer.” (Risch et al., 1988)] Hoover and Strasser (1980) reported increased relative risk with heavy and prolonged use of tabletop artificial sweeteners and diet drinks, among non-smoking women not in high-risk occupations, and among male heavy smokers. Cartwright et al. (1981) reported increased risk for “takers” of saccharin, compared with non-takers, but only among male non-smokers.

In the National Cancer Institute study, the reported increased relative risk with heavy and prolonged use was based upon only seven individuals out of a study population of 3,000 bladder cancer patients. As noted by the authors, this “study was designed to include large numbers of subjects in order to assess the effect of just such heavy use.” (NCI report, p. 26)\* (Heavy use was defined as six or more servings a day of a sugar substitute or two or more eight-ounce diet beverages a day.) “However, there were few heavy users of both types of AS, so that there was substantial variability in the RR’s for heavy use, and none of the RR’s in Table 10 by itself would be statistically significant.” (NCI report, p. 17) The authors also state “these excesses were relatively small in epidemiologic terms” . . . “did not show a consistent dose-response relationship” . . . and “were based on relatively small numbers of subjects” (less than 2% of the population studied). (NCI

\*Only those references with an asterisk are provided with these comments. All others accompanied the Calorie Control Council’s 1996 petition to NTP.

report, p. 25) “In addition, the pattern of positive RR’s described (at high dose, and particularly evident in a low-risk subgroup and among heavy users) could be ascribed to chance variation in subgroups of a study which, overall, shows no association between AS use and bladder cancer.” (NCI report, p. 31) Table 10 [Exhibit IV] from which the finding of a reported increased risk comes, also shows two of the heaviest artificial sweetener user groups had reported decreased risks. For example, an individual who drinks less than 2 cans of diet soda and uses between 3 and 6 servings of tabletop sweetener per day has a 24% decreased risk of bladder cancer. The authors further point out, “The inconsistencies associated with the relatively small increases in risk among heavy users suggest caution in their interpretation and a need for further analyses.” (NCI report, p. 26) It is evident from the above statements that little confidence can be placed in the reported finding of an increased risk for heavy users of artificial sweeteners. Additionally, those using AS for the longest number of years and using the most had relative risks of .96 and 1.05, respectively (Tables 13 and 12).

The increased relative risk of bladder cancer among non-smoking women not in high-risk occupations reported by Hoover and Strasser is another example of very small sub group data being used to formulate interpretations extended to the general population. For instance, statistical significance for low-risk women is generated in Table 14 [Exhibit III] of the NCI report to a large degree by the fact that 9 of 15 cases had reported use of tabletop sweetener. Had the proportion been 8 of 16, the result would not have been statistically significant. That is, changing the exposure of 1 person would eliminate the statistical basis of the data on which the allegations rest.

The low-risk” group of white females was allegedly chosen to evaluate the potential effects of a weak carcinogen. Because the numbers in this “low-risk” group were too small to examine dosage, the criteria for definition of “low risk” was relaxed to include coffee drinkers, [Thus this group was not established *a priori*.] It is notable that the investigators chose to base their interpretations on as few as 80 cases out of a possible 3,000 and relaxed their criteria to include a potentially confounding variable, but did not choose to include non-white females, nor discuss any other low-risk group as a basis for this type of estimate. In the closest comparable subgroup of low-risk males for which data are provided (i.e., male non-smokers), there was no increased risk for artificial sweetener users versus non users. It is important to note that, at the time of the Hoover and Strasser report, the controversial Canadian epidemiologic study (Howe et al. 1977) had suggested an association between tabletop sweeteners and the incidence of bladder cancer only among male users, and the proportion of males in the NCI study outnumbered females 3 to 1.

Although Hoover and Strasser reported an increased relative risk of bladder cancer use among male heavy smokers who used artificial sweeteners there appears to be a decreased

RR of bladder cancer for artificial sweetener users. It was only those males who smoked 40 plus cigarettes per day for whom the apparent risk of bladder cancer was greater with the use of artificial sweeteners than without. And in this group, the dose/response relationship is inconsistent. The fact that increased risk (Table 18) is apparently associated with artificial sweetener use only in the heaviest smoking category suggests that residual confounding due to the established relationship of cigarette smoking to bladder cancer, not artificial sweeteners, is responsible for such increased risk. We agree with the NCI investigators who state:

“Further analyses will be needed to describe fully the relationships of risk with AS among heavy smokers. Interpretation of these findings should also be aided by analyses of other groups at high risk because of extensive exposure to other bladder carcinogens.” (NCI report, p. 23)

Interestingly, three studies -- two subsequent to the NCI report -- have reported statistically significant protective associations with artificial sweeteners. Morrison et al. (1982) noted a decreased risk among both sexes with a history of sugar substitute use while Morgan and Jain (1974) and Moller-Jensen et al. (1983)\* reported a decreased risk in women only with prolonged use of any type of artificial sweetener and in men only with use of diet drinks.

Epidemiologists recognize that if any study, especially large studies which lend themselves to subdivision, is partitioned into numerous sub samples, there may be a problem of so-called ‘significant’ associations occurring by chance with multiple comparisons. Although it may be difficult to distinguish chance associations from the causal “because of the inconsistencies between studies in identifying special groups (they differ by both sex and risk-factor category), along with the equally frequent ‘protective’ effect, there is a strong suspicion that the associations reported (positive or negative) are by chance, rather than causal.” (Morgan and Wong, 1985)

Contrary to a statement made at the October 31 meeting that “none of the epi studies have looked at *in utero* exposure of saccharin,” Jensen & Kamby (1982)\* examined the issue of *in utero* exposure to saccharin by examining bladder tumor incidence among persons born in Denmark between 1941-45, a time of high saccharin use. There was no increase in risk in cohorts exposed to saccharin as long as 35 years prior to the study.

Illustratively, the question was raised about the stage of malignancy with increased saccharin consumption as reported by Sturgeon et al. (1994).\* This observation was based on just five cases - such a small number that only one case could control the conclusion.

Dr. Zahm stated “that really epidemiology has only focused on bladder cancer.” This statement is not entirely accurate. Kessler (1970) reviewed the mortality of 21,447 diabetics (a group which uses considerable amounts of artificial sweeteners) followed from 1930 to 1959 and found no increase in deaths from cancer. Because cyclamate was not

widely available until the 1960's, the sweetener consumption for this cohort was likely limited to saccharin. Additionally, Morrison (1979)\* found that an age-sex-country-standardized estimate of cancer incidence for users of artificial sweeteners showed no increased cancer risk. The study concluded: "The present data provide virtually no support for an overall positive association of AS with cancer."

### **Dose/Consumption**

The Calorie Control Council's petition to NTP provided an abundance of data on the consumption of saccharin, including consumption by children. To summarize, the results of three studies conducted in North America were included: the 1977-78 USDA Nationwide Food Consumption Survey, the 1986 MRCA Information Services survey and the Nutrition Canada Food Consumption Patterns Reported (undated & unpublished). The youngest age group surveyed and the average calculated saccharin intakes in mg/kg/day for these survey were 1-2 years, 11.46; 1-4 years, 11.22; and 2-5 years, 0.44; respectively. The wide discrepancy between the MRCA data and those in the other two surveys reflects differences in collection and analytical techniques. Furthermore, the MRCA data are based on actual food intake; the other surveys assume that each of the food groups containing saccharin are consumed every day -- a highly unlikely event. Actual diet soft drink saccharin intake values today are even lower than the 0.44 mg/kg/day reported in the MRCA survey. In the MRCA survey it was assumed that all diets soft drinks consumed were sweetened with saccharin, while today most are sweetened with aspartame alone with the exception of fountain drinks which are generally sweetened with a combination of aspartame and saccharin. With FDA's additional approvals of aspartame and acesulfame potassium the number of food and beverage products containing saccharin and the per capita consumption of saccharin has decreased further. And, the recent approval of sucralose may result in an even further decrease.

Even the highest intake level for children reported in the three surveys, 11.46 mg/kg/day, which does not reflect true intake, is 130 times less than the lowest, daily, lifetime intake of sodium saccharin associated with bladder tumors in the rat.

Saccharin consumption studies also have been conducted in Australia, Finland, the United Kingdom, Germany, The Netherlands and Denmark. The data span the period from when saccharin was the only intense sweetener approved in some countries to present times when there are often a number of sweeteners available and they may be used alone or in blends. Intakes of saccharin have been shown to be below the acceptable daily intake (ADI) (0-5mg/kg/day) set by JECFA in all studies for the mean and 90th percentile intakes. Studies in Australia and the European Union (Germany, The Netherlands and Denmark) did not find individuals with 7-day averages above the ADI. Intake by the 97.5 percentile in young children (less than 5 years of age) in the United Kingdom slightly exceeded the ADI because of the intake from the consumption of diluted juice concentrates (products that are not nor have ever been available in the U.S.). (Renwick, 1995\*)

## **Animal studies**

As noted above, the Council petitioned NTP to delist saccharin on the basis of NTP's new criteria for delisting which provide for the consideration of data indicating that the agent acts through mechanisms which do not operate in humans and would therefore reasonably be anticipated not to cause cancer in humans. There is an abundance of data supporting sodium saccharin's mode of action on the male rat bladder which was provided in the Council's petition. Unfortunately, there was very little reference to this data by the NTP presenter at the October meeting.

To reiterate, mechanistic studies of the bladder tumors in male rats fed high dietary levels of sodium saccharin have demonstrated that changes in urine physiology and hyperplastic alterations in the urothelium occur over the same sodium saccharin dose range associated with bladder tumors. Changes observed include increased urinary sodium levels, high pH and low osmolality. (Arnold et al., 1980a, b; West and Jackson, 1981; Renwick and Sims, 1983; Schoenig and Anderson, 1985; Anderson et al., 1988; Fukushima et al., 1988a; Garland et al., 1989a, 1991a, 1991b; Fisher et al., 1989; Shibata et al., 1992; Shioya et al., 1994; Cohen, 1995a). Coincident with these changes, increased urinary crystalluria and a flocculent precipitate are also present. (Chowaniec and Hicks (1979); Taylor et al., 1980; Arnold et al., 1980a & b; West and Jackson, 1981; Schoenig et al., 1985; Cohen et al., 1995a, b, c). The particulates observed in the urine of male rats fed sodium saccharin were much less marked in female rats and did not occur in mice (Arnold et al., 1980a, b; Arnold et al., 1995). Crystalluria and precipitates do not occur in rats fed high dose levels of sodium saccharin if the pH of the urine is decreased by feeding the sodium saccharin in the AIN-76A diet or by adding  $\text{NH}_4\text{Cl}$  to the diet (Cohen et al., 1995a; Cohen et al., unpublished observations). Crystals and precipitates in rats fed sodium saccharin are associated with the male rat protein  $\alpha_{2u}$ -globulin (Eklund et al., 1992). The involvement of this protein provides an explanation for the predominance of bladder tumors in the male rather than the female rat fed sodium saccharin (Cohen et al., 1995b).

Studies using the two-stage bladder tumor initiation/promotion model have shown that the urinary physiological changes that occur in the rat fed high dietary levels of sodium saccharin are obligatory for bladder tumor promotion. (Nakanishi et al., 1980a; Fukushima et al., 1983a; Hagiwara et al., 1984). Studies using this model have also shown that bladder tumor promotion occurs when a variety of sodium salts of organic acids are fed to the rat including ascorbic, citric, erythorbic and glutamic acids. Bladder tumor promotion does not occur when the corresponding parent acid is fed (Fukushima et al., 1983b; Fukushima et al., 1986a, c; Fukushima et al., 1984; deGroot et al., 1988). These studies have provided evidence that the bladder tumors that develop in male rats fed sodium saccharin or other sodium salts are the result of a non-specific process that is dependent on urinary pH and sodium levels and the presence of particulates in the urine. It has been shown, in agreement with this hypothesis, that feeding acid saccharin to the rat does not cause the urinary or bladder changes that occur when sodium saccharin is fed and does not promote the development of bladder tumors (West et al., 1986). This difference occurs even though urinary saccharin levels are comparable in each case.

The results of the IRDC study of sodium saccharin in the rat, as reported by Schoenig *et al.* (1985), demonstrated a NOEL at a dietary level of between 1 and 3%. Squire (1985) found that a 3% dietary level of sodium saccharin was a NOEL in this study. The threshold dietary level of 2.7% sodium saccharin calculated by Carlborg (1985) is considered to be a reasonable approximation of the NOEL for sodium saccharin. This 2.7% threshold level far exceeds any amount that could conceivably be consumed by humans and is equivalent to a daily consumption of approximately 620 12-ounce diet soft drinks (sweetened with sodium saccharin alone at 9.2 mg per ounce).

The second generation male rat was early on identified as the most sensitive sex and species and was consequently examined more thoroughly (Taylor *et al.*, 1980; Tisdell *et al.*, 1974; Arnold *et al.*, 1980a). Thus, more rigorous saccharin studies have been done in male rats than female rats or in male or female mice. But studies have been carried out not only in female rats, male and female mice (including a three-generation carcinogenicity study) but also in hamsters and monkeys (Arnold *et al.*, 1980a; Kroes *et al.*, 1977; Althoff *et al.*, 1975; Takayama *et al.*, 1998\*). It is now widely accepted among the scientific community that sodium saccharin does not pose a carcinogenic risk for humans. Saccharin is approved in more than 100 countries around the world. In its Information Letter of Dec. 5, 1991\* Canada's Health Protection Branch stated that "the majority view of toxicologists is that saccharin, at low doses, does not pose a health risk for humans". It has been reviewed and determined safe by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993) and the Scientific Committee for Food of the European Union (SCF, 1994). Based on current research, JECFA has increased the ADI (acceptable daily intake) for saccharin. JECFA concluded at their forty-first meeting that "on the basis of data reviewed to date, it would be inappropriate to consider the bladder tumors induced in male rats by sodium saccharin to be relevant to the assessment of a toxicological hazard to humans".

Dr. Maronpot and some Board members failed to note that there is an abundance of data on the effects of sodium saccharin in the female rat providing substantial support for the quantitative differences between the changes that occur with the ingestion of high doses of sodium saccharin in the male versus the female rat and there were no adverse saccharin related findings in other species, including no proliferation or other effects on the urine or bladder in any of these studies (see references for other species above). Although the urinary parameters of the male and female rat exposed to high doses of sodium saccharin are essentially the same through 6-8 weeks of age there is a significant difference as they mature. The differences between mature male and female rats are explained by the large amounts of  $\alpha_{2u}$ -globulin in the urine of the mature male rat (Cohen *et al.*, 1995b). There is an extensive literature on these differences which was referenced in the Council's petition to NTP.

Dr. Maronpot questioned the meaning of urinary parameters observed in rats fed high doses of saccharin which developed bladder tumors. Although the exact role of increased urine volume and low osmolality has not been defined it was shown in the IRDC bioassay (Schoenig *et al.*, 1995) that rats within the F<sub>1</sub> generation 7.5% sodium saccharin treatment

group which developed bladder tumors showed significantly greater urine volume and lower osmolality than animals which did not. From this and numerous other experiments it is clear that the development of bladder tumors in rats fed high doses of sodium saccharin is a multi-factorial process and three critical points are urinary volume, osmolality changes and bladder distention. Furthermore, the differences in urine pH are not the important issue but in order for tumors to develop, the urine pH must be 6.5 or above (Fukushima et al., 1986; Garland et al., 1989a).

Dr. Maronpot stated that "Urinary bladder carcinogenesis has been demonstrated in mice following urinary bladder implants of cholesterol pellets that contain saccharin." With Dr. Mirer's (October 31 transcript, p. 61) further discussion of the implantation studies Dr. Frederick, a fellow Board member, noted "those have clearly fallen from favor in bladder cancer research" and carefully explained why. There is now general scientific agreement that urinary bladder pellet studies are not useful in determining the human risks of bladder cancer. For example, these, as well as a number of other, direct bladder exposure studies have been assessed by the Mitre Corporation under a contract with the Food and Drug Administration. The reviewers concluded that "Both types of direct bladder exposure studies, pellet implantation and intravesicular catheterization, are considered unsuitable for predicting human carcinogenic risk." (The Mitre Corporation, 1987, p. 6-4).\*

In addition, Dr. Maronpot emphasized one study by Prasad and Rai (1986b) in male and female mice in which thyroid tumors were observed. In this study, male and female mice fed 1.5 g/kg saccharin (1.0% of diet) for one year had papillary adenocarcinomas of the thyroid. The tumors were present in 5/10 males and 3/10 females as large protuberant masses beneath the skin. In the absence of confirmatory data it is doubtful that these tumors can be attributed to saccharin. Other authors (Roe et al., 1970; Miyaji, 1977; Homburger, 1978; Frederick et al., 1989) fed saccharin to mice for periods of 18 to 30 months at dietary levels up to 5%. Although thyroids are not mentioned specifically in these publications, it is not credible that the authors would have missed the type of lesions described by Prasad and Rai. Furthermore, Dr. Frederick noted that in a large saccharin mouse study conducted at the National Center for Toxicological (NCTR), research in which saccharin doses ranged from 0.1 percent to 5.0, the thyroid was examined and no effects on the thyroid were observed. (October 31 transcript, p. 35)

Furthermore, according to the Office of Technology Assessment 1977 publication *Cancer Testing Technology and Saccharin*, extensively referenced by Dr. Jacobson without OTA's conclusion, "To conclude, the two generation experiments show that saccharin causes an increase in bladder cancer in second generation animals, especially among males. In the one experiment in which the first generation was also examined, the increase fell just short of the standard test of significance. No cancer of any other sight has been convincingly associated with saccharin." (The Council was surprised at some Board members' apparent acceptance without question of comments presented by Michael Jacobson. OTA discounted a number of the studies he cited.) Dr. Clay Frederick also noted at the October 31 meeting in the NCTR mouse study over 40 organs were examined without positive results.

Dr. Hooper stated: "In rodents, in the rat studies, in looking at concentrations, the fetus binds four to five times as much in the bladder as the maternal animal does." Saccharin research does not support this statement. Sweatman and Renwick (1982) have reported the tissue levels of saccharin in the rat during two-generation feeding studies. A single oral dose of saccharin was given to female rats in late pregnancy. The concentrations in rat fetal tissue 6 to 12 hours after dosing were lower than those in the mother. However, the concentrations in the fetal tissues, including the bladder wall, decreased more slowly, so that by 48 hours they exceeded the corresponding values obtained for maternal tissues, suggesting the possibility of accumulation during chronic intake. Despite this, the steady-state concentrations of saccharin in the liver and kidneys of fetuses from mothers fed 5% saccharin diet ad libitum were lower than the corresponding maternal values, while the concentrations in the fetal bladder were similar or slightly higher. The concentrations of saccharin in the tissues of rats in utero were not markedly higher than those found in adult F<sub>1</sub> animals. The turnover of saccharin in the fetuses of animals maintained on a 5% saccharin diet was similar to that seen after a single dose.

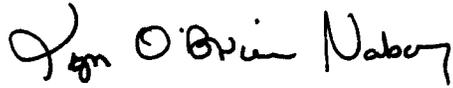
Dr. Hooper questioned the correlation of the urinary precipitate to tumors stating that the NOEL for the labeling index was 0.1 percent saccharin diet per Murasaki & Cohen (1981) which is lower than the dose for the precipitate, that there was no precipitate in the 1957 skin painting studies, and that there was no precipitate in the Milo et al. (1988) MNU study in cultured human fibroblasts or the implant studies. First, a calculation of 0.1 percent is based on only four animals in the 0.1 percent group and the controls. In this experiment in which 0.1 percent sodium saccharin in the diet led to an increased labeling index, the mean was increased on the basis of an increase in only one animal. The other three were well within control levels. More recent studies have shown that an occasional rat bladder urothelium, even controls, has a similarly increased level of labeling index and that the handling of animals alone actually increases the labeling index in the bladder of both control and saccharin fed rats. Furthermore, current recommendations are that a minimum of 10 animals per group for evaluation of labeling index be utilized to avoid the vagaries of individual animal variation.

Second, in a number of incidences saccharin has been used to test the test, as in the MNU study in cultured human fibroblasts. In this study, malignant transformation occurred when culture human foreskin fibroblasts were exposed to non-toxic concentrations of sodium saccharin during the G<sub>1</sub> release phase of mitosis followed by MNU or ENU. Transformation frequency was not affected by saccharin alone and "cocarcinogenicity" did not occur if saccharin treatment occurred after exposure to MNU. The relevance of such data to the human experience is highly questionable as is the skin painting. In ingestion studies, saccharin does not cause skin cancer. The implant studies have been discussed above.

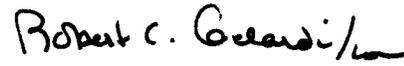
**Conclusion**

In conclusion, the totality of the saccharin research overwhelmingly supports its safety for human consumption and its delisting from NTP's Report on Carcinogens.

Sincerely,



Lyn O'Brien Nabors  
Executive Vice President



Robert C. Gelardi  
President

LONRCG/jac  
Enclosures