Safety of saccharin
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ABSTRACT: Long term treatment with high doses of sodium saccharin in the diet beginning in utero or at birth induces urinary bladder tumours in the rat with a higher incidence in male rats compared to female rats. After one of the most extensive toxicological evaluations of any chemical, it ultimately was shown that saccharin-induced bladder cancer in rats is a species-specific high dose phenomenon, and it is now accepted that saccharin does not pose a cancer risk to humans. The methodology utilized and the issues investigated in the research with saccharin have greatly extended the sciences of toxicology, epidemiology and risk assessment for chemicals.

INTRODUCTION
Saccharin has been the focus of controversy nearly since its initial synthesis in 1879 by Fahlberg and Remsen. Its primary use has been as a non-caloric synthetic sweetener, but it is also being used commercially for a variety of other purposes including as an antiseptic, preservative, nickel-plating, antistatic agent, and numerous other uses. It is an inexpensive, extremely stable chemical that is resistant to heat. Numerous toxicological effects have been attributed to saccharin, dating to its initial proposed use as a sweetener. There is the famous quote by President Theodore Roosevelt in response to being told that saccharin was “injurious to health” by Dr. Harvey W. Wiley of the US Department of Agriculture: “You tell me that saccharin is injurious to health? Why my doctor gives it to me everyday. Anybody who says saccharin is injurious to health is an idiot”. Most of the various accusations regarding the toxicity of saccharin were addressed over the years, with its commercial use continuing. In 1970 a new controversy swirled around the use of saccharin as an artificial sweetener with the discovery that it produced bladder cancer when administered to rats in a two-generation bioassay. Significant concern ensued that it might also pose a cancer risk to humans. Addressing this controversy has extended the limits of toxicological testing and mechanistic considerations, with results not only influencing the decision regarding saccharin but also setting precedence for numerous other chemicals in various settings, not just food additives. For a history of saccharin, including the more recent controversy involving bladder cancer, the readers are encouraged to read several reviews on the subject (1-4).

Animal testing is the foundation for toxicological screening of chemicals for nearly all uses, including food additives. There are two basic assumptions when using animal models for such screening (2, 5): 1) the toxic effect produced in the test species will also occur in humans (species extrapolation); and 2) the effect seen at the doses used in the screening evaluation are relevant to the levels to which humans are exposed (dose extrapolation). For many chemicals and toxic endpoints, these assumptions are reasonable. However, it is only with mechanistic understanding that one can address either of these assumptions. For the influence of saccharin on the bladder in rats, based on extensive mechanistic research, neither of these assumptions were found to be valid. It is a rat specific phenomenon and it only occurs at extremely high doses (25,000 ppm of the diet and higher).

SACCHARIN CARCINOGENICITY STUDIES
Saccharin is one of most intensely investigated chemicals regarding its possible carcinogenicity, including multiple rodent bioassays, extensive epidemiology investigations, and mechanistic evaluations (2-5). For the most part, standard one-generation bioassays in rodents have been negative if administration begins at the usual age of 4-8 weeks. Based on a suggestion by Dr. Leo Friedman at the US Food and Drug Administration (FDA), saccharin was one of the first chemicals to be evaluated in a two-generation bioassay (1). This involves administration of the test chemical to the dams prior to conception, continuing during gestation and lactation, and then continuing administration to the offspring (F1) generation for a period of two years after weaning. In two-generation bioassays, saccharin produced an increased incidence of bladder tumours in rats, particularly in male rats. The results generally have not been statistically significant in female rats, but tumours and preneoplastic proliferative lesions have occurred in females. The initial two-generation bioassay was performed by the Wisconsin Alumni Research Foundation (WARF), and was repeated by the FDA. There were concerns in these two studies regarding procedural aspects as well as the purity of the saccharin, including contamination with o-toluenesulfonamide (OTS). This led to a study performed in Canada which was designed to address these problems and was to be utilized as the “ultimate” test for a regulatory decision regarding the carcinogenicity of saccharin. Based on the Canadian study, it was concluded that the bladder cancer effects in rats in a two-generation bioassay were due to the saccharin and were not due to OTS or procedural inadequacies in the testing. Based on these results, Canada banned the use of saccharin for dietary uses, and the US FDA proposed a similar ban. Because of an outcry from the public, since the only other artificial sweetener, cyclamate, had already been banned in the United States, Congress passed a moratorium delaying the ban by the FDA until additional research could be completed. A fourth two-generation bioassay was performed at the International Research and Development Corporation (IRDC) laboratory involving a large number of animals so that a detailed dose response could be determined (6). In addition, the effects of administration in utero compared to post-gestational exposure was evaluated. The study examined only the male offspring. They were able to show that there was no significant effect at a dose below 3 percent of the diet. Furthermore, they were able to show that beginning
administration at the time of birth produced the same results as exposure beginning prior to conception, through gestation and onward. This clearly indicated that the effect was a high dose phenomenon, and also indicated that the effect was due to exposure after birth, not due to in utero exposures. While the two-generation studies were being performed in the 1970’s there were also several investigations examining saccharin as a so-called “tumour promoter” (2, 4). This involved administering saccharin in the diet after the rats had been pre-treated with a known, potent, DNA reactive bladder carcinogen. Under these circumstances, high doses of saccharin (usually 5 percent or more of the diet, but positive results were obtained with doses of 2.5 percent of the diet or higher) yielded a high incidence of bladder tumours. Long term (usually 2 years) studies were positive when the rats had been pretreated with one of a variety of potent, known bladder carcinogens, such as N-methyl-N-nitrosourea (MNU), N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (FANFT), or N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), but not 2-acetylaminofluorene (2-AAF). These studies involved administration to animals beginning at 4-8 weeks of age or older. In the groups administered only sodium saccharin, bladder tumours were either not detected or were not present at statistically significant incidences. These studies were designed to follow the initiation-promotion protocols originally developed in mouse skin and later in rat liver.

Thus, by the end of the 1970’s, there was considerable evidence that saccharin administered in the diet (but not in the drinking water) increased the incidence of bladder cancer in rats, particularly in males. By this time there was considerable effort to investigate the mechanism that was involved in producing these rat bladder tumours so that the possible risk to humans could be rationally assessed.

MECHANISTIC STUDIES OF SACCHARIN-INDUCED CARCINOGENICITY IN THE RAT

Mechanistically, a chemical can increase the risk of cancer either by direct DNA damage (DNA reactivity) or by increasing cell proliferation (5). Increasing cell proliferation can either be by increasing cell births or decreasing cell deaths with a corresponding accumulation of cells. Extensive investigations have been performed addressing these possibilities. In all of the bioassays in rats, the form of saccharin that was administered was the sodium salt. Saccharin is a moderately strong acid with a pKₐ of 1.8 (2, 4). The acid itself is quite insoluble in aqueous media. Commercially, one of the salts is used for dietary use, usually sodium, but calcium saccharin has also been used.

The initial clue that led to the ultimate demonstration of the mechanism producing urothelial cytotoxicity was an observation that the effects on the urinary bladder and on the urine composition following the administration of different salts or acid form of saccharin were considerably different (2). Thus, the urothelial toxicity and proliferative response to sodium saccharin was the greatest, followed by potassium saccharin. A small but statistically insignificant change occurred in response to calcium saccharin, and no effect was seen with acid saccharin. The amount of saccharin in the diet was the same for each of these, and the amount of saccharin excreted in the urine was also the extent the influence on pH of the medium. Following the discovery of these effects by saccharin salts, it has become standard practice to evaluate these two parameters in any in vitro genotoxicity evaluation to avoid a false positive interpretation of the test (7). Based on the results of these genotoxicity assays, saccharin is considered not to be genotoxic, and more specifically, it is not considered DNA reactive. DNA reactivity is highly unlikely given the anionic nature of the saccharin ion, lack of metabolic conversion, and lack of any reactive site that is available for its interaction with DNA. Furthermore, an in-vivo in vitro assay evaluating the unscheduled DNA synthesis following saccharin administration was negative as was examination for interaction of saccharin covalently with the DNA of the urothelium of the bladder, the target tissue (2, 4). Thus, the mode of action of saccharin effects on the urinary bladder epithelium of the rat must involve an increase in cell proliferation. Such an increase was demonstrated utilizing tritiated-thymidine or bromodeoxyuridine (BrdU) incorporation as methods for assessing DNA replication (2). The labelling index is increased following dietary administration of sodium saccharin by 2-10 times compared to the control bladder urothelium, which is a slowly proliferating tissue (turnover of approximately 100-200 days). The increased proliferation was shown to be due to a regenerative response to cytotoxicity involving the superficial cell layer of the urothelium. Since the superficial cells of the urothelium can be extremely thin, it can be difficult to demonstrate cytotoxicity using routine light microscopic examination. By examining the entire luminal surface of the urinary bladder by scanning electron microscopy (SEM), the multifocal, superficial nature of the cytotoxicity, necrosis, and exfoliation could be demonstrated along with the early proliferative changes. Thus, the determining factor for saccharin-induced, rat bladder carcinogenesis was the process by which cytotoxicity was induced.

The saccharin anion is not metabolized by mammalian organ­isms (1, 2). Thus, whatever is ingested is excreted intact. Most is excreted in the urine in rodents as well as in humans. The genotoxicity of saccharin has been examined extensively in a variety of in vitro and in vivo assays (1, 2, 4). It has generally produced negative results in the in vitro studies and has been uniformly negative in the in vivo assays. In vitro it has produced positive results in a few assays, but confounding factors were ultimately identified which caused the apparent positive response. These effects included primarily the high ionic strength and osmolality that is required with the salts and to a lesser extent the influence on pH of the medium. Following the discovery of these effects by saccharin salts, it has become standard practice to evaluate these two parameters in any in vitro genotoxicity evaluation to avoid a false positive interpretation of the test (7). Based on the results of these genotoxicity assays, saccharin is considered not to be genotoxic, and more specifically, it is not considered DNA reactive. DNA reactivity is highly unlikely given the anionic nature of the saccharin ion, lack of metabolic conversion, and lack of any reactive site that is available for its interaction with DNA. Furthermore, an in-vivo in vitro assay evaluating the unscheduled DNA synthesis following saccharin administration was negative as was examination for interaction of saccharin covalently with the DNA of the urothelium of the bladder, the target tissue (2, 4). Thus, the mode of action of saccharin effects on the urinary bladder epithelium of the rat must involve an increase in cell proliferation. Such an increase was demonstrated utilizing tritiated-thymidine or bromodeoxyuridine (BrdU) incorporation as methods for assessing DNA replication (2). The labelling index is increased following dietary administration of sodium saccharin by 2-10 times compared to the control bladder urothelium, which is a slowly proliferating tissue (turnover of approximately 100-200 days). The increased proliferation was shown to be due to a regenerative response to cytotoxicity involving the superficial cell layer of the urothelium. Since the superficial cells of the urothelium can be extremely thin, it can be difficult to demonstrate cytotoxicity using routine light microscopic examination. By examining the entire luminal surface of the urinary bladder by scanning electron microscopy (SEM), the multifocal, superficial nature of the cytotoxicity, necrosis, and exfoliation could be demonstrated along with the early proliferative changes. Thus, the determining factor for saccharin-induced, rat bladder carcinogenesis was the process by which cytotoxicity was induced.
same regardless of the form of saccharin that was administered. However, there were dramatic differences in concentrations of the normal urinary constituents, especially the pH. Following administration of acid saccharin, the urinary pH is <6.0, whereas following the administration of sodium or potassium saccharin the urinary pH was >6.5, usually 7.0 or above. Calcium saccharin produced urinary pH that varied slightly above or below 6.5. Based on the significant changes in urinary composition following sodium saccharin administration at high doses, a calcium phosphate-containing precipitate forms in the urine (2-5, 8-10). This precipitate also contains silicate, mucopolysaccharide, protein, and low quantities of the saccharin anion (<5 percent). All of these substances except the saccharin are normal constituents of rat urine. Calcium and phosphate are particularly important for numerous biological functions. As long as they are present at sufficiently low concentrations they remain in solution and are not toxic to cells; they are actually required for normal cellular functions. However, when the concentration rises sufficiently high so that the threshold for solubility is exceeded, the calcium phosphate precipitates in the urine and becomes cytotoxic. It has been shown with numerous types of epithelial cells that calcium and phosphate are required for viability, but when the concentration reaches the level where precipitation occurs, the calcium phosphate-containing precipitate is toxic and lethal to the cells. The critical nature of this precipitate for the cytotoxic, proliferative, and tumorigenic effects of saccharin could be demonstrated by administering the saccharin in situations where the urine could be acidified below pH 6.5 (2-4, 8). A pH of 6.5 is the minimum level in the rat urine which is required for calcium phosphate to precipitate. If the pH is below 6.5, the precipitate does not form; there is no evidence of cytotoxicity, consequent regeneration, or tumour formation. Acidification of the urine can occur either by administration of saccharin as the acid itself, administering sodium saccharin in AIN-76A diet which produces a highly acidic urine, or co-administering sodium saccharin with ammonium chloride in the diet which also leads to marked acidification of the urine. The dose response for the formation of the urinary precipitate demonstrated that it formed only at extremely high concentrations of saccharin in the diet, generally greater than 25,000 ppm of the diet or greater (2, 4, 6). Thus, the assumption of high to low dose extrapolation in the rat is not valid; it is a high dose phenomenon only with a true threshold. The same threshold level occurs for the cytotoxicity, regenerative proliferation and tumorigenicity. No biological effects on the urothelium are detected when high concentrations of sodium saccharin are administered to mice, even concentrations as high as 10 percent of the diet (2, 4). Under these circumstances, even with administration of sodium saccharin and of urinary pH above 6.5, there is no formation of calcium phosphate-containing precipitate. In contrast to the rat, the mouse has a significantly lower level of calcium and phosphate as well as magnesium in the urine; 10 times or greater calcium concentration is present in rat urine compared to mouse urine. Since the solubility of calcium phosphate is dependant on the concentrations of calcium and phosphate, these significant differences in their urinary concentration are sufficient to explain why calcium phosphate-containing precipitate does not form in the mouse. Saccharin incorporated into either paraffin or cholesterol pellets inserted directly into the bladder lumen of mice was shown to increase the risk of bladder cancer (2, 11). However, the saccharin was extremely rapidly eluted from these pellets (half-life of less than a day) so that the exposure in the bladder was both brief and at very low levels. It was ultimately shown that the rapid elution of saccharin from these pellets resulted in a very coarse pellet. It is the course pellet that produced the effects on the bladder urothelium, a phenomenon that was described by Clayson in 1976 for calculi in general (12). Administration of saccharin in the diet to mice produced no effect on the urothelium of the bladder. Humans and non-human primates are able to produce urine with a urinary pH above 6.5 and with urinary concentrations of calcium and phosphate as high as seen in the rat (2, 4, 13). However, the rat also has extremely dense urine because of high ionic concentrations and extremely high concentrations of urea. The osmolality of rat urine is 1500 mosmol or greater. In contrast, human urine is usually below 400 mosmol even under conditions of dehydration. Furthermore, rat urine contains extremely high concentrations of protein (mg/ml) whereas primate and human urine generally contains significantly lower concentrations of protein (µg/ml). The generation of calcium phosphate-containing precipitate is contingent on several parameters being at appropriate levels (8-10, 13). This appears to only occur in the rat, and more so in the male than the female rat. The male rat has
much higher concentrations of protein due to the presence of α₂u-globulin compared to the female. The NBR strain of rats does not produce the same high levels of α₂u-globulin in the males as in other strains of rats. In the NBR strain of rats, sodium saccharin produces the same slight effect in the male as in the female, considerably less of a response than seen in male rats of other strains with α₂u-globulin in large quantities. If it is the high exposures of primarily the sodium (and to a lesser extent the potassium) salt of saccharin that produces these effects, can sodium and potassium salts of other moderately strong acids also produce a similar effect? The answer is an emphatic yes (8–10, 14). A large number of sodium salts have been tested for formation of calcium phosphate-containing precipitate, urothelial cytotoxicity and increased proliferation as well as tumour promoting activity. One of these sodium salts, sodium ascorbate (vitamin C), has also been evaluated in a two-generation bioassay. The exception is sodium hippurate, which does not produce a proliferative or tumour promoting effect in the rat bladder (10, 14). As frequently occurs in biology, it is exceptions like this that help define specific mechanisms. For reasons that are unclear, doses of sodium hippurate comparable to the carcinogenic dose of sodium saccharin, in contrast to the other sodium salts, produces a urinary pH in the rat of <6.5. Since this is a critical pH level for formation of the calcium phosphate-containing precipitate, it is expected that sodium hippurate does not have an effect on the bladder epithelium and that is what is found. Several sodium salts have been evaluated for one or more of the effects that are involved with sodium saccharin bladder carcino genesis in the rat, including formation of the calcium phosphate-containing precipitate, superficial cytotoxicity with consequent regenerative proliferation, tumour promotion, or tumour induction in a two-generation bioassay (3, 4, 10). These are listed in Table 1. Saccharin is the only synthetic sodium salt that is on this list, the others are natural and many of the substances on this list are essential ingredients in the diet and all of them, except saccharin, are essential for normal cellular functions. Based on mechanistic considerations, both of the assumptions stated at the beginning of this article, dose extrapolation and species extrapolation, are not valid for the urinary bladder effects of sodium saccharin in the rat. It is a high dose phenomenon that occurs only in rats.

EPIDEMIOLOGIC STUDIES OF SACCHARIN

Extensive epidemiologic investigations of saccharin have also been performed, primarily in diabetic populations, including one of the largest case control studies ever performed (3, 4, 15). The mechanistic considerations described above predict that saccharin ingestion by humans would not be a risk factor for cancer in general, or bladder cancer specifically. This has been corroborated by a lack of increased risk in epidemiologic investigations. Based on the mechanistic details described above, a panel of the International Agency for Research on Cancer (IARC) was convened to evaluate the species specificity of the calcium phosphate-containing precipitate mechanism for producing bladder cancer in rats (3). It was unanimously supported by the worldwide scientists convened at that meeting. Based on that evaluation, a year later the IARC down classified saccharin from its previous classification as 2B (possibly carcinogenic to humans) to 3 (not classifiable as to its carcinogenicity to humans, based on mechanism) (4). Furthermore, based on the mechanistic considerations and epidemiology, the National Toxicology Program of the United States removed saccharin from its congressionally mandated List of Carcinogens in 2000. The down classification of saccharin by IARC and its removal from the List of Carcinogens were precedent setting. The mechanisms and process by which these events occurred have been followed in subsequent evaluations of other chemicals. Later, Congress removed the warning label that had been mandated for tabletop packages of saccharin for use as a sweetener.

SUMMARY

Saccharin has had an extremely long and controversial record since its synthesis in 1879, continuing until recently. It is one of the most extensively toxicologically evaluated chemicals. The issue concerning bladder cancer ultimately was shown to be a high dose phenomenon that occurred only in rats. The methodology and issues utilized in the research with saccharin have greatly extended the sciences of toxicology, epidemiology and risk assessment for chemicals. It is now accepted that saccharin does not pose a cancer risk to humans.

REFERENCES AND NOTES

7. D. Kirkland, S. Pfuhler et al., "How to reduce false positive results when undertaking in vitro genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop", Mutat. Res., 628, pp. 31-55 (2007).