RESEARCHES REGARDING THE TOXICOLOGY OF SACCHARIN

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ABSTRACT

Saccharin, first synthesized in 1879, eventually became popular as an inexpensive substitute for sugar, particularly as a non-caloric sweetener. The dispute is concerning the safety of saccharin itself. In this article, the history concerning the uses of saccahrin and the accompanying controversy is reviewed. A benefit-risk evaluation for saccharin showed few, if any documentable benefits from the use of saccharin and much genuine uncertainty concerning the potential risks for ingestion by man. This element of genuine uncertainty as to the extent of human risk posed to man is the crux of saccharin's past and its foreseeable future.

1. INTRODUCTION

Saccharin was discovered accidentally in 1879 by Constantin Fahlberg while working in Ira Remesen's laboratory at John Hopkins University. The controversy regarding the safety of saccharin for human consumption was well under way by 1890 and has not appreciably abated in the intervening years.

Saccharin's commercial importance and much of its notoriety is primarily attributable to its use as an artificial, non-caloric sweetener. However, some of the original uses of saccharin were as an antiseptic and preservative to retard fermentation in food and it was found to be useful in estimating the circulation time of blood from an antecubital vein to the lingual capillaries; It was also used as a brightener in nickel-plated automobile bumpers, an antistatic agent in plastics and textiles, a polymer modifier and accelerator in photosensitive dispersions, and a light-fastness aid in nylon dyes; it serves as a chemical intermediate for the fungicide probenazole in Japan and it is a chemical of research interest in other disciplines such as nutrition and several areas of psychology.

2. MATERIALS AND METHODS

Prior to 1959, several toxicological studies of short and intermediate duration had been conducted with different species of laboratory animals and man, but only one chronic/carcinogenic bioassay had been undertaken.

Fitzhugh et al. reported in 1951 the results of their 2-year feeding study in which groups of 16 to 20 Osborne-Mendel rats received a control diet or diets containing 1% or 5% sodium saccharin. While there was slight growth suppression in the 5% group, saccharin had no apparent effect upon mortality, hematology, or the weight of the liver, kidney, or spleen. The only significant pathological change was observed in the 5% group, where seven animals had thoracic lymphosarcomas and four of these seven rats had abdominal lymphosarcomas as well. Even though the strain of rats used in this study had a high spontaneous incidence of lymphosarcomas, the authors stated that the incidence observed in this study was unusually high. The urinary bladders were not

examined in this study. Although the authors concluded that saccharin caused only slight toxic effects in the 5% group, the evaluation of the pathological changes observed in this study is still being debated.

The second saccharin chronic/carcinogenic bioassay was reported by Lessel in 1970. This study used groups of 20 male and 20 female Wistar rats fed diets containing 0, 0.005, 0.05, 0.5 or 5% saccharin for 2 years. Another group received 1 ml of a 1% solution of trypan blue sc, every 2 weeks for 1 year as a positive control. Mortality was higher in the 5% and trypan blue groups, and retardation of growth despite a greater feed consumption was observed in the 5% group. Four males and one female rat in the 5% group had bladder calculi and another male had kidney calculi. The female rat with calculi had an extensive transitional cell papilloma of the bladder while another 5% female without calculi had hyperplasia and a papilloma. No evidence of nematodes was found. Historically, this study has been repeatedly cited by those who have hypothesized that the ingestion of saccharin by laboratory rats elicits the production of bladder stones. In several experiments with rats, bladder stones have been found to increase the incidence of urinary bladder tumors.

The first two-generation bioassay was apparently conducted with saccharin. This study contained groups of 20 male and 20 female Sprague-Dawley rats fed diets containing 0.0, 0.05, 0.5 or 5.0% sodium saccharin prepared by the Remsen – Fahlberg procedure. These diets were administered for 14 weeks prior to mating, and then during mating and lactation. Subsequently 20 pups per group were weaned onto their respective parents' diet which they consumed for 100 weeks. Following weaning, the F_0 generation was killed. Consequently, the F_1 animals were exposed to saccharin and its impurities during gestation, throughout lactation via the dam's milk and for the remainder of their lifetime via the diet.

The significant toxicological observations made on the F_1 animals included:

1) Saccharin treatment had no remarkable effects upon the reproductive indices except for a depressed pup body weight in the 5.0% group at weaning; however, by 13 weeks after weaning, the growth curves were similar in all groups for the balance of the study.
2) Neither survival nor feed consumption were affected by treatment and the observed hematological changes were primarily attributed to poor health.
3) The authors suggested that the incidence of tumors was apparently increased in the 5% group, particularly the incidence of squamous cell carcinomas of the uterus and transitional cell carcinomas of the urinary bladder. These two tumor types were seen exclusively in the treated groups, with higher incidence at higher dosage levels.

The second two-generation feeding study in which the toxicological effects of saccharin were studied was conducted by the US Food and Drug Administration. This study was a combined three-generation reproduction study and two-generation chronic bioassay. The F_0 portion of this study consisted of groups of 10 male and 20 female Sprague-Dawley rats being fed diets containing 0.0, 0.01, 0.1, 5.0, or 7.5% sodium saccharin produced by the Remsen-Fahlberg procedure. One additional group was fed a diet containing 1.51% sodium carbonate, which resulted in a diet having the same sodium content as 5.0% sodium saccharin. A second group was fed a diet containing 5.0 % calcium cyclamate, which was fed as a reference compound. A total of 57 F_1 males and 57 F_1 females were randomly selected from each group. There were interim kills after 14 months, using four rats/sex/group, and after 18 months, using five

rats/sex/group. The study was terminated after 109 to 121 weeks post weaning. The F_0 animals were killed after the F_1 animals were weaned.

Important toxicological findings included the following:

1) Neither hematological values, organ weights, nor survival were affected by the treatment.
2) Birth weights of rats in the 5.0 or 7.5% saccahrin or 5.0% cyclamate groups were depressed versus controls. While some of these initial body weight differences were overcome, the animals in these test groups had a lower body weight throughout the study.
3) A total of nine urinary bladder neoplasms were found in the 7.5% group (seven males, two females), one in a 5.0% male and one in a control male. In addition, sections of bladder from the dome, midpoint, and bladder neck from each rat that was on test for more than 18 months were examined for evidence of the bladder parasite *T. crassicauda*. As no evidence of parasites was found, the authors concluded that parasites did not contribute to the tumor incidence nor did the incidence of gross calculi.

3. DICUSSIONS AND RESULTS

To focus on events happening outside the animal laboratory for a moment, the apparent inability of the one-generation studies to elicit a tumorigenic response was consistent with the findings of a number of epidemiological studies that were reported in the scientific literature during the early seventies. Additionally, the high dosages of saccharin required to elicit bladder tumors in the two-generation studies, as well as the preponderance of bladder tumors in the second generation males, produced more questions than answers for the toxicological problems being studied. However, these findings did suggest that the toxicological manifestations attributed to saccharin in the two-generation bioassays might well be the result of one or more impurities in saccharin produced by the Remsen-Fahlberg procedure. Examination of what role impurities might have in this problem was first examined by chemists in the Health Protection Branch of Health and Welfare Canada.

They found that ortho-toluenesulfonamide (o-TS), was the major impurity in all of the lots of saccharin used in the various long-term feeding studies. The concentration of o-TS in the lots of saccharin used in these studies was found to range from 118 to 6100 ppm. These and other observations led to the hypothesis that o-TS a known inhibitor of carbonic anhydrase, th enzyme involved in the acidification of urine, would result in an increased excretion of bicarbonate. As a consequence, an alkaline urine could result, predisposing the animal to urolithiasis in the kidney and bladder; irritation of the bladder wall by these calculi could in time produce hyperplasia and ultimately tumors. A correlation between the presence of bladder stones and bladder tumors has previously been found for rats during several experimental situations, but a similar set of events does not result in pathological lesions in human urinary bladders.

Upon reviewing all of the toxicological data concerning saccharin, it was concluded that sodium saccharin itself rather than any impurity was most likely responsible for the induction of the bladder tumors observed in rats. The extrapolation of animal data to man is subject to a considerable amount of biological and statistical uncertainty, with any direct experimental demonstration of a threshold or no-effect dose for carcinogenesis being virtually impossible in the absence of knowledge regarding the mechanism involved. Consequently, the fact that habitual use and even conceivable

intakes of saccharin by man were considerably below the animal dosage shown to be carcinogenic in the two-generation bioassays, by no means excluded the possibility that saccharin might be a human carcinogen in practice as well as in theory.

It was well recognized that saccharin was used commercially not only as a cheap substitute for sugar, but also to replace sugar in certain foods particularly to replace sugar in carbonated beverages that were consumed by diabetics and others who wanted a low-calorie drink with a similar degree of sweetness to the sugared product. Consequently, the question as to whether or not saccharin was unique, or beneficial, for man regarding the treatment of obesity, the maintenance of ideal weight, or of significant assistance in the maintenance of the health or quality of lifestyle for the diabetic, was considered. The scientific literature regarding this question prior to March 1977 was scant and did not support the existence of these purported benefits. Additionally, results from some animal studies have suggested that saccharin may even be a stimulant for increased feed ingestion , but others have reported contrary observations.

While there was little doubt that saccharin per se was the agent responsible for the bladder tumors observed in all three two-generation animal feeding studies, it was also recognized that further research was required regarding several aspects of this problem.

Saccharin has had a very colourful and interesting history. It is the most thoroughly investigated food additive tested in the two-generation chronic/carcinogenic bioassay, the first food additive which has been the subject of several epidemiological studies, and the only food additive for which moratoriums were instituted to forestall a ban.

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