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Bladder Tumors in Rats Fed Cyclohexylamine or High Doses of a Mixture of Cyclamate and Saccharin

Abstract. *Papillary transitional cell tumors were found in the urinary bladders in 8 rats out of 80 that received 2600 milligrams per kilogram of body weight per day of a mixture of sodium cyclamate and sodium saccharin (10 : 1) for up to 105 weeks. From week 79 on, several of these rats received cyclohexylamine hydrochloride (125 milligrams per kilogram per day, the molecular equivalent of the conversion of about 10 percent of the cyclamate dosage to cyclohexylamine) in addition to the sodium cyclamate and sodium saccharin. In another study in which 50 rats were fed daily 15 milligrams of cyclohexylamine sulfate per kilogram of body weight for 2 years, eight males and nine females survived. One of the eight males had a tumor of the urinary bladder. In neither study were bladder tumors found in the control rats or in rats treated with lower doses of the compounds.*

Numerous requests have been made for the information which was presented to the National Academy of Sciences–National Research Council (NAS–NRC) ad hoc Committee on Nonnutritive Sweeteners on 17 October 1969, and which led to the order by the Secretary of Health, Education and Welfare that cyclamates be removed from the list of substances generally recognized as safe (GRAS). In this preliminary report we present the pertinent experimental findings in the context of some relevant historical information.

The enactment of the Food Additives Amendment of 1958 made it necessary to establish at least a partial list of substances generally recognized as safe since such substances generally were exempted from the application of this statute. Food and Drug Administration (FDA) scientists prepared such a list, which included cyclamates, and this was sent to over 900 qualified scientists for comment. Of the 355 scientists who responded, only one commented on cyclamates stating that he was unfamiliar with the data on these sweeteners. Thus, cyclamates were included in the published list, as set forth in the Code of Federal Regulations (Section 121.101).

In 1962, the Food and Nutrition Board of the NAS–NRC issued a revised policy statement which said that artificial sweeteners could be safely used in limited amounts as a nonnutritive substitute for sugar in special purpose foods.

In 1965 and again in September 1967, scientists of the FDA reexamined all available information about cyclamates and concluded that there was no evidence that the amounts of cyclamates then being used presented a hazard to health. In 1967, the joint FAO/WHO Expert Committee on Food Additives established an acceptable daily intake of 50 mg of cyclamate per kilogram of body weight. In 1968, the NAS–NRC recommended the limitation of daily intake to be 70 mg per kilogram of body weight. On the basis of these two reviews, in April 1969, the FDA proposed steps to achieve revised product labeling that would limit the daily intake to the level recommended by WHO.

The above reviews included an examination of studies in which rats were fed diets containing 1 and 5 percent saccharin or sodium cyclamate for 2 years. These compounds produced no effects at the lower dose and no distinct toxic effects at the high dose (*1*). Toxicological studies in rats fed diets containing 1 and 2 percent sodium cyclamate for periods up to 11 months indicated no significant adverse effects of this compound (*2*).

Allen *et al.* (*3*) reported in 1957 that surgical implantation of pellets containing 4 parts of cholesterol and 1 part of saccharin into the urinary bladder of mice induced one papilloma and three carcinomas of the bladder among 13 animals that survived 40 to 52 weeks. In 1966, a similar study with sodium cyclamate was initiated by one of us (J.M.P.) at the University of Wisconsin. On 5 June 1969, a preliminary verbal report (*4*) of this study was given to Abbott Laboratories, stating that a significant incidence of bladder tumors had been found in white Swiss mice in two separate experiments with the pellet implantation technique. Representatives of Abbott Laboratories had several discussions about these findings with representatives of the National Cancer Institute and the Food and Drug Administration during June and July. It was the judgment of all concerned that tests for carcinogenicity by the pellet implantation technique (*3*) were not suitable for evaluating the hazard of orally ingested compounds. A similar position regarding data obtained by this technique had been taken by the NAS–NRC ad hoc Committee on Nonnutritive Sweeteners in 1968. Plans for additional toxicity studies of cyclamates, cyclohexylamine (CHA), and saccharin were then agreed upon. It was also decided to pay special attention to the urinary bladders of rats in two toxicity studies sponsored by Abbott Laboratories which had been initiated in 1967 and were nearing completion.

One of the last-mentioned experiments, conducted at Industrial Bio-Test Laboratories, Northbrook, Illinois, was a 2-year toxicity study of cyclohexylamine in rats which was designed to ascertain whether or not the CHA which could be present in minute amounts in commercial cyclamates might be toxic. Charles River strain albino rats in groups of 25 males (125 g) and 25 females (123 g) were given daily doses of either 0, 0.15, 1.5 or 15.0 mg of cyclohexylamine sulfate per kilogram of body weight. During the first year of the study, there was only a slight depression in the weight gain curves observed in male animals fed the highest dose (*5*). There were no significant differences between test and control animals as to food consumption, mortality, blood chemistry, or hematologic parameters. At the end of 2 years, eight males and nine females were alive in the high dose group. There were 13 to 16 survivors in each of the other three groups at the end of the study. No drug-related changes were found in any of the organs examined except in the urinary bladder. A bladder tumor was found in one of the eight male survivors in the high dose group which was diagnosed as invasive transitional cell carcinoma, grade 2. The tumor did not invade the muscular wall of the bladder, and no metastatic lesions

Table 1. Summary of the preliminary data obtained in the long-term feeding study of sodium cyclamate and sodium saccharin (C/S). At the 79th week groups B, C, and D were each divided into two subgroups each containing approximately half the surviving number of converters and nonconverters. Subgroups 1 and 2 continued to receive C/S at the stated dose and subgroup 2 received in addition the indicated dose of CHA (the molecular equivalent of the conversion of about 10 percent of the cyclamate to CHA).

Group	Daily dose (mg/kg day)		No. of animals alive at week								No. converters†/ No. tested		No. tumors‡	
			0		56*		78		104					
	C/S	CHA	M	F	M	F	M	F	M	F	M	F	M	F
A	0	0	35	45	25	35	20	35	13	26			0	0
B	500	25	35	45	25	35	20	30	10	19	11/23	5/33	0	0
C	1120	56	35	45	25	35	20	31	8	23	9/24	9/32	0	0
D	2500	125	35	45	25	35	20	30	12	22	23/25	32/35	7	1

* Ten males (M) and ten females (F) died or were killed for interim study by the 56th week. There was one death in each group except for group B females (none) and group D males (two). † Rats excreting CHA in the urine in amounts equivalent to more than 0.1 percent of the cyclamate fed (see text). ‡ Urinary bladder tumors agreed upon by all of the pathologists on the basis of the slides available to date. Four to eight of these tumors were diagnosed as carcinomas by different pathologists.

were present. Spontaneous bladder tumors have never been recorded in control rats at Industrial Bio-Test Laboratories (*5*) or at Abbott Laboratories and are reported to be very rare (*6*).

The second experiment, conducted at Food and Drug Research Laboratories, Maspeth, N.Y., was a 2-year toxicity study of a 10 : 1 mixture of sodium cyclamate and sodium saccharin (C/S) which was added to the diet of Wistar strain rats in concentrations providing a daily intake of 0, 500, 1120, or 2500 mg per kilogram of body weight (Table 1). The concentrations required to provide the stated daily doses of the mixture were determined from data obtained by biweekly weighing of the animals and biweekly measurements of their food intake. The rats were maintained throughout the 2-year period in individual cages in air-conditioned and humidity-controlled quarters, with water and food freely available.

During this study many of the rats were found to convert cyclamate to cyclohexylamine (*7*). The rats were considered to convert cyclamate to cyclohexylamine if more than 0.1 percent of the cyclamate was accounted for as urinary CHA. The extent to which individual rats converted (or whether they converted) was variable. The maximum conversion rate was 12.6 percent (*7*).

In the 79th week, one-half of the animals in each of the treated groups were given supplemental amounts of cyclohexylamine hydrochloride mixed in the diet and calculated (as the base) to provide daily intakes of 25, 56, or 125 mg per kilogram of body weight. All major organs and tissues, including the urinary bladder, were examined histologically in the surviving animals as well as in those animals that died or were killed in the course of the study. Among the 240 rats receiving C/S, seven males and one female of the group fed 2500 mg per kilogram per day showed papillary tumors of the urinary bladder (Table 1) which were diagnosed by seven pathologists (*8*). In all but one instance, the tumors developed in rats that had been found to convert cyclamate to CHA. There were three bladder tumors in animals that received supplemental CHA and five in those that did not. Macroscopically, tumors were seen in only two animals. Of the eight tumors, four to eight were diagnosed as carcinomas by the different pathologists. No gross bladder calculi were found in the eight rats with tumors. Three of the tumors were found between weeks 78 and 83, and the remaining tumors were found in animals which were killed between 100 and 105 weeks of the study.

On 8 October 1969, Abbott Laboratories was first notified by telephone of the presence of bladder lesions in rats fed the C/S mixture. On 9 October Abbott pathologists observed the presence of bladder tumor in one of the rats fed CHA. On 13 October Abbott representatives reviewed the microscopic slides and other data from the study of the C/S mixture at Food and Drug Research Laboratories and on the same day reported the findings to scientists of the National Cancer Institute. On 14 October these findings were discussed in a joint meeting of representatives of Abbott Laboratories, the National Cancer Institute, FDA, and the Department of Health, Education and Welfare, and it was decided to report the findings to the NAS–NRC ad hoc Committee on Nonnutritive Sweeteners. The slides of the urinary bladders of the rats from the two studies were reviewed on 15 and 16 October by additional staff and consultant pathologists of the National Cancer Institute. All the available data from these experiments were presented on 17 October to the NAS–NRC Committee which recommended the removal of cyclamates from the GRAS list.

The development of bladder neoplasms had not been reported in other species or in other strains of rats fed cyclamate or saccharin. There is no evidence that the use of cyclamate or saccharin has caused cancer in man, malformations in children, or any other abnormality in humans other than a rare skin hypersensitivity. However, in view of the requirements of the Delaney clause of the Food Additives Amendment, the removal of cyclamates from the classification of substances generally recognized as safe resulted in the prohibition of their use in general purpose food products.

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