CHAPTER I

INTRODUCTION

Saccharin was first synthesized by Remsen and Fahlberg (1879), who discovered the sweetness of this compound in the course of an academic investigation at Johns Hopkins University, on the oxidation of o-toluenesulfonamide. Since that time many other synthetic sweeteners have been investigated but only a few have been used commercially. Saccharin has an intensely sweet taste led to the comprehensive investigation of the manufacture.

The sweetness of saccharin varies from 200 to 700 times that of sucrose depending upon the method of determination and individuals, for most tasters it has a sweetening power about 300 times of sucrose. It has no food value and is used when reduction of the consumption of sugar is desirable (Noller, 1952).

Saccharin has many synonyms as follows : 2,3-dihydro-3-oxobenzisosulfonazole; 1,2-benzisothiazol-3(2H)-one 1,1-dioxide; 1,2-dihydro-2-ketobenzisosulfonazole; saccharin insoluble; benzosulfimide; o-sulfobenzimide; benzoic sulfimide; o-sulfobenzoic acid imide; Garantose; Glucid; Gluside; Glusidum; Hermesetas; Saccharinol; Saccharinose; Saccharol; Saxin; Sykose (Stecher, 1968).

Saccharin ($C_7H_5NO_3S$) has the formula weight of 183.18, occurs as white crystals or white crystalline powder, odorless or with faint aromatic odor, melting point 228.8°-229.7°C. The sweet taste of saccharin is still detecable in 1 : 100,000 dilution. Saccharin in excessive concentration tends to display a bitter note to the taste. The exact concentration at which this effect occur varies with individuals. Saccharin threshold occurs in vicinity of 0.1 per cent which is above the level of ordinary use (Kirk and Othmer, 1954).

The solubility of saccharin is as follows : one gram in 290 ml of water, 25 ml of boiling water, 31 ml of alcohol, freely soluble in solution of alkali carbonates, slightly soluble in chloroform, ether. It possesses acid reaction, pH of 0.35 per cent aqueous solution is 2.0 (Stecher, 1968).

Saccharin is stable under all conditions ordinarily encountered in food preparation and processing, and no loss of sweetening power nor development of off flavors due to instability is encountered. For all practical purposes, saccharin in aqueous buffered solutions of pH 3.3, 7.0, and 8.0 are uneffected by heating at temperature up to 150°C for one hour (DeGarmo et al. , 1952).

Saccharin decreases the sourness of hydrochloric acid to about the same extent that sucrose does. It is further

interesting to note that the relative sweetness increases with increasing concentration of alcohol in aqueous-alcohol solutions. The sweetness of saccharin is also increased by the presence of another sweetening agent, dulcin. Dulcin and urea diminish the unpleasant aftertaste of saccharin. It has been claimed that one-half or more of the sucrose used in making up a sweetened foodstuff may be replaced by saccharin before the taste of the latter becomes noticeable. The cyclamate-saccharin mixtures were commonly used in the production of a wide variety of good-tasting foods and beverages for diabetics to consume from 1965 to 1969. However, if the product contains organic acids and is boiled, hydrolysis occurs to a sufficient extent to change the taste (Suter, 1945). The hydrolysis products of saccharin are o-sulfamoylbenzoic acid (alkaline hydrolysis) and ammonium o-sulfobenzoic acid (acid hydrolysis) (DeGarmo et al. ,1952).

Actually, hydrolysis occurs with water alone, although the reaction is slow. Boiling a water solution results in 97.4 per cent hydrolysis in 96 hours, and at room temperature 17.6 per cent hydrolysis in 166 days. The sodium salt is more stable, hydrolysis amounting to only 6 per cent at 230° C in 2 hours (Suter, 1945).

The structure of saccharin (1) makes many types of reaction possible. The imido hydrogen is acidic, and therefore, saccharin forms salts with bases. Many metallic salts have

been prepared, not all of which are sweet. The copper salt is astringent, the nickel salt is only slightly sweet while calcium, sodium, and ammonium salts are intensely sweet. Chlorination of saccharin sodium in aqueous solution yield 2-chlorosaccharin. Saccharin sodium will react with a great variety of halogen compounds to give N-alkyl or aryl-substituted saccharin (2) (Kirk and Othmer, 1954). Saccharin condenses with phenols to gives saccharéins of type (3) (Richter, 1945). Structure of saccharin and its derivatives are shown in Figure 1.

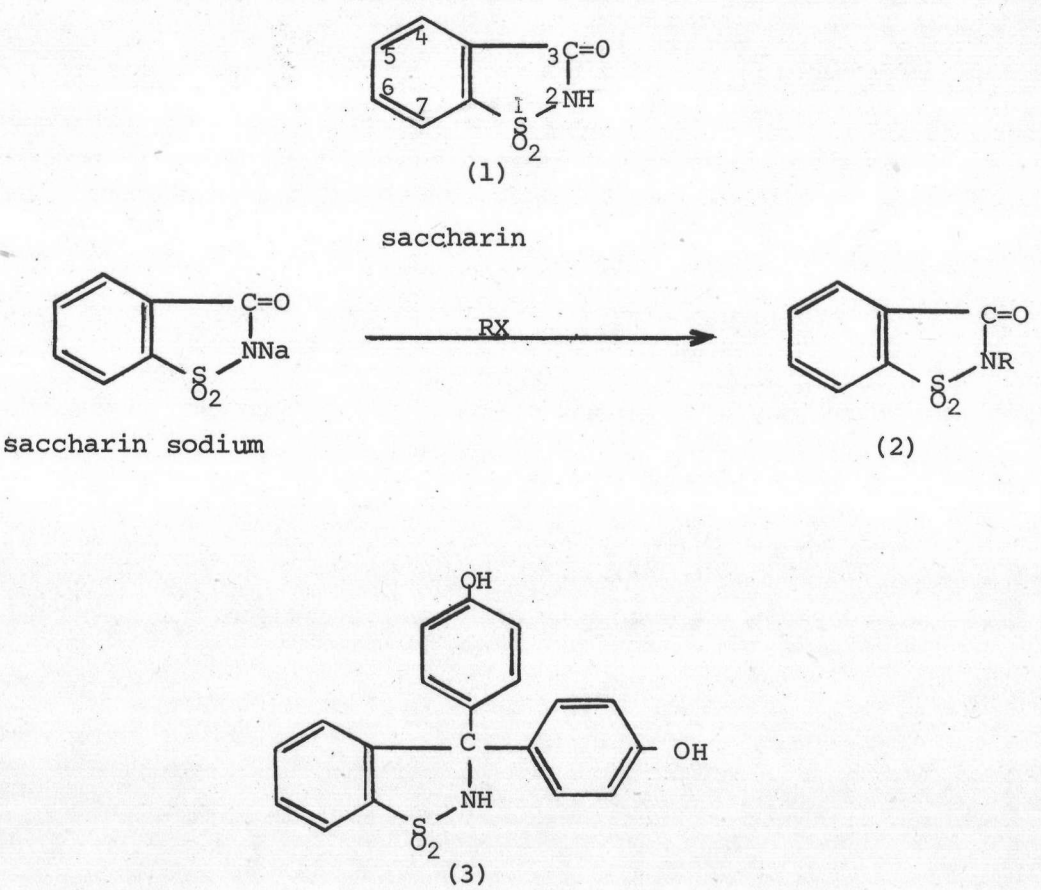


Figure 1. Structure of saccharin and saccharin derivatives.

The most important methods for preparing saccharin depend upon o-toluenesulfonamide as the starting material, the same method used by Remsen and Fahlberg (1879). Toluene and chlorosulfonic acid react at 0°-5°C to form a mixture of o- and p- toluenesulfonyl chlorides, which are converted by means of ammonia to a mixture of o- and p- toluenesulfonamides. The mixture is separated, and the o-toluenesulfonamide is oxidized to o-carboxybenzenesulfonamide (o-sulfamoylbenzoic acid). This compound loses a mole of water to become saccharin, as in Figure 2. (Kirk and Othmer, 1954).

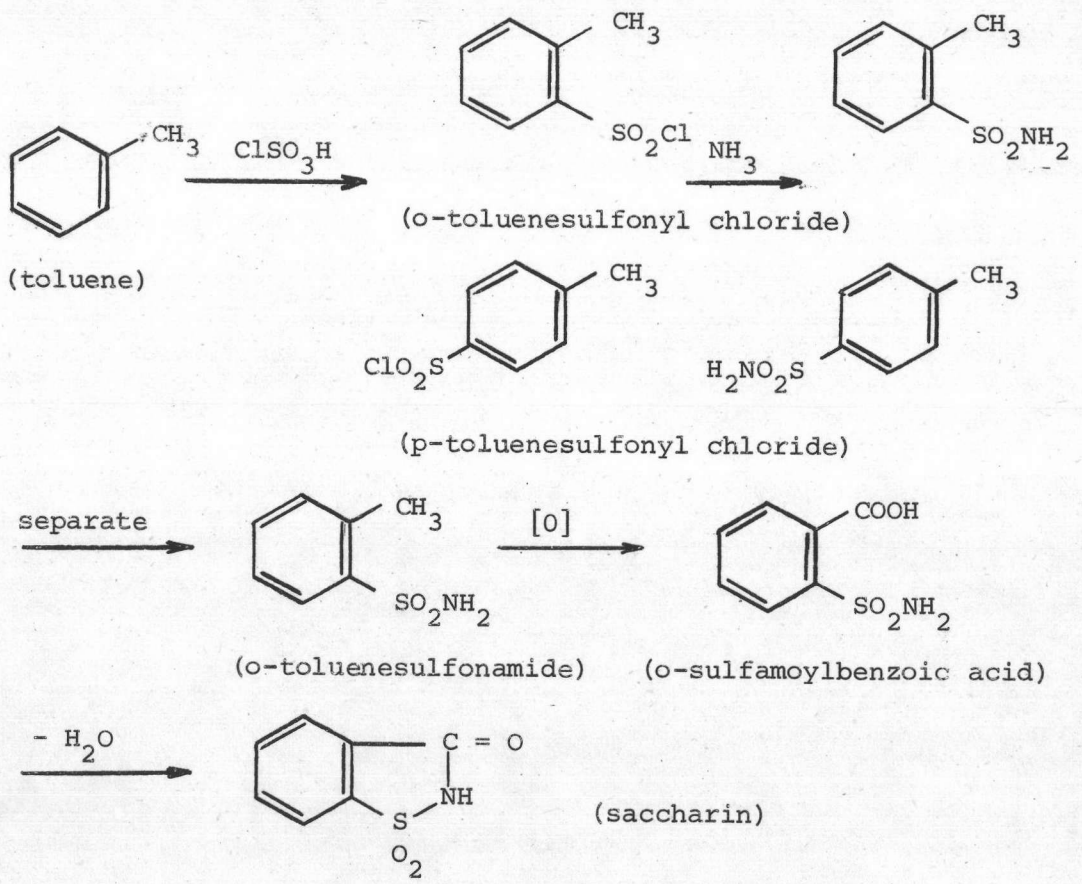


Figure 2. Synthesis of saccharin from o-toluenesulfonamide.

Many modifications of this basic process, particularly of the oxidation step, have been proposed so as to achieve the best possible yields. Orelup (1926) used chromic acid mixed with sulfuric acid at over 50 per cent concentration, and this is the method most widely used today.

Altwegg and Collardeau (1924) found the addition of sulfates of iron, chromium, or manganese to be helpful. Best results are obtained at 50°-60°C with 70 per cent sulfuric acid, the yield of saccharin approximating 90 per cent of the theoretical amount. Löwe (1922) used an electrolytic method in which o-toluenesulfonamide is suspended in a weak alkali carbonate. This reaction is favored by the presence of lead, cerium, or manganese. Klages (1919) used an electrolytic process with addition of permanganate and alkaline potassium ferricyanide have also been employed. A synthesis of saccharin based on anthranilic acid which is used commercially in the United States, is shown in Figure 3. (Kirk and Othmer, 1954).

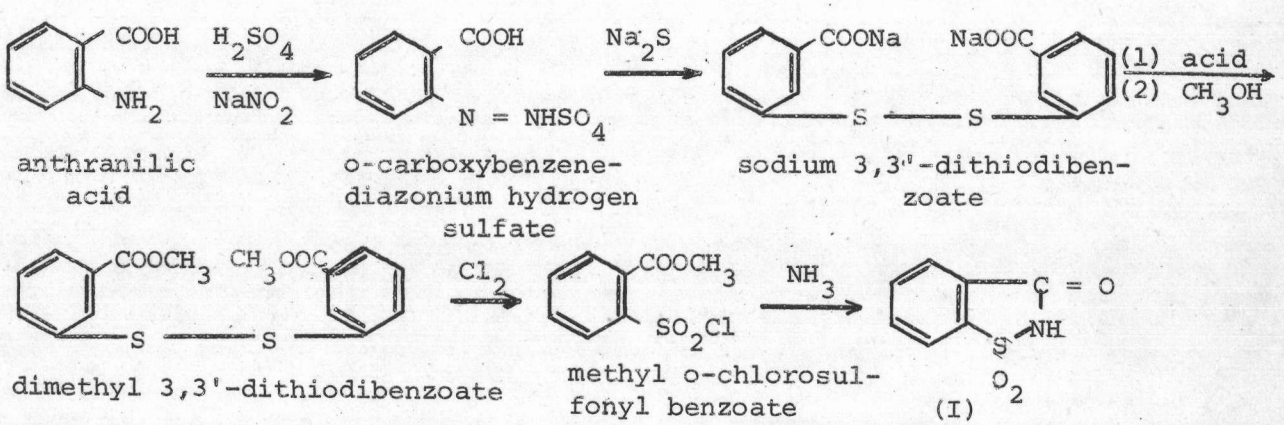
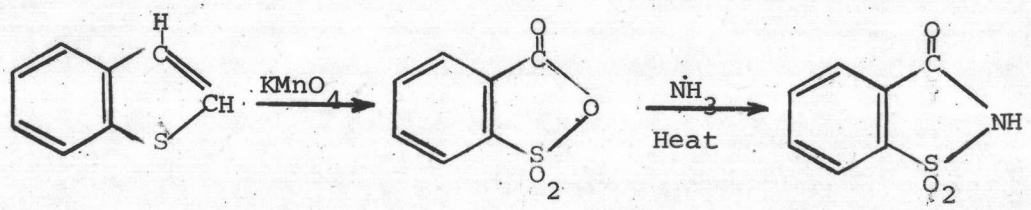


Figure 3. Synthesis of saccharin based on anthranilic acid.

Numerous other syntheses of saccharin have been devised and some of these have been used commercially in various parts of the world (Dalal and Shah, 1950).

It is reported that the slightly bitter taste associated with saccharin is caused by the presence of o-toluamide. Moreover the disposal of p-toluenesulfonyl chloride obtained as a by product in the process shown in Figure 2. has been a problem. Both of these objections are overcome by two methods, one starting with anthranilic acid as mentioned in Figure 3., and the other, starting with thianaphthene (Noller, 1965) shown in Figure 4.



thianaphthene saccharin

Figure 4. Synthesis of saccharin starting with thianaphthene

The impurities suggested over the years as possibly present in saccharin manufactured by oxidation of o-toluenesulfonamide were listed by King and Wragg (1966) and by Rader et al. (1967). The impurities that might be visualized are : o-sulfamoylbenzoic acid; p-sulfamoylbenzoic acid; o-toluenesulfonamide; p-toluenesulfonamide; ammonium o-sulfobenzoate; ammonium-

p-sulfobenzoate; saccharin-o-toluenesulfonylimide; toluene-2,4-disulfonamide; and saccharin-4-sulfonamide. The first four are the most usual impurities seen in small but variable amounts in commercial saccharin. The last three are rarely detected in saccharin if at all. The various commercial saccharin synthesized by Remsen and Fahlberg (1879) process contained o-toluenesulfonamide (OTS) as an impurity in concentration ranging from 118 to 6,100 ppm. But the process started with phthalic anhydride, the OTS impurity amounts to only 1 to 3 ppm in the commercial product. At the present time the main focus of attention is on OTS as the major impurity (Coon, 1975).

Saccharin derivatives, free of toluenesulfonamide impurities, prepared by the reaction of 2-sulfobenzoic acid derivatives with $COCl_2$ in dimethyl formamide (DMF), reported by Koike et al. (1977) gave 82.4 per cent saccharin of 99.8 per cent purity.

After the discovery of the non-caloric sweetener in 1879, saccharin began its history, which has been regularly punctuated by debate regarding its safety. As early as 1907, this debated reached such proportions that the President of the United States appointed a review board to evaluate the safety of saccharin. World War I and World War II were the periods of high consumption, particularly in Europe, but usage decreased in the post-war period when sugar was again available in adequate supply (Tisdel et al., 1974).

It was first used in 1880 as an antiseptic and food preservative, and in the mid 1880 was first used by diabetics. In 1912, a board of scientific advisors of the United States concluded 300 milligram of saccharin per day was safe and that one gram per day may cause digestive disturbances. The latter amount is the FDA recommended limit for the daily consumption of saccharin (Coon, 1975).

Not only is saccharin used as a sweetening agent for those, such as diabetics, who need a low sugar diet but also in time of carbohydrate scarcity, it may come into more general use. It has been reported that in Germany during 1917-1919, about 1,000 tons of artificial sweeteners replaced some 200,000 tons of sugar. At about the same time it was recommended for use in Italy in order to save sugar for children, invalids, and soldiers (Suter, 1945).

The introduction of the combination of saccharin and calcium cyclamate provided a more satisfactory product and this, coupled with an increasingly diet-conscious public, resulted in a rapid increase in consumption of non-nutritive sweeteners. Because the newer sweetener (cyclamate) had less history of usage and was present at ten times the level of saccharin, the debate on safety shifted from saccharin to calcium cyclamate. In 1969, cyclamates were banned from use as artificial sweeteners so increased demand of saccharin, which again became the center of the debate on the safety (Tisdel et al. , 1974).

When the safety of saccharin was reviewed in 1970, the Food Protection Committee (FPC) of the National Academy of Sciences of the United States recommended :

(1) long-term feeding studies in two species of animals, according to modern protocols to determine chronic toxicity with particular attention to pathological examination of the kidney and urinary bladder for carcinogenic hazard;

(2) metabolism studies in man; and (3) studies of toxicological interaction between saccharin and certain drugs. Long-term chronic toxicity studies conducted in several laboratories during the period 1958 to 1970 are shown in Table 1. (Zienty, 1974).

Table 1

Chronic toxicity studies on saccharin, 1958-1970

(After Zienty, 1974)

Investigator	Species	Product Form*
Dr. B. Lessel (1970) Boots Pure Drug Co., Ltd. Nottingham, England	Rat	S
Dr. O. Garth Fitzhugh et al. (1951) Food and Drug Administration Washington, D.C.	Rat	SS
Dr. F.J.C. Roe et al. (1970) Chester Beatty Research Institute London, England	Mouse	SS

* S = Saccharin (free acid)

SS = Sodium saccharin

Accordingly, extensive chronic toxicity studies were undertaken in the laboratories listed in Table 2. (Zienty, 1974).

Table 2

Chronic toxicity studies on saccharin, 1970-1974

(After Zienty, 1974)

Investigator	Species	Product Form*
Dr. L. Golberg Institute of Experimental Pathology Albany Medical College Albany, New York	Rhesus - Monkey	SS
	Rat	SS
Dr. B. Lessel Boots Pure Drug Co., Ltd. Nottingham, England	Rat	S
Dr. I.C. Munro, Dr. H. Grice Health Protection Branch Ottawa, Canada	Rat	SS
Dr. P. Shubik Eppley Institute for Research on Cancer Omaha, Nebraska	Hamster	SS
Dr. L. Friedman Food and Drug Administration Washinton, D.C.	Rat	SS
	Hamster	SS
Prof. D. Schmähl German Cancer Centre Heidelberg, Germany	Rat	SS

* S = Saccharin (free acid)
SS = Sodium Saccharin

Table 2. (Continued)

Investigator	Species	Product Form*
Dr. T. Miyaji National Institute of Hygienic Sciences Medical Department Osaka University, Japan	Rat	SS
Dr. J.H. Weisburger National Cancer Institute Washington, D.C.	Mouse Rat Rat	SS SS S
Dr. G.J. Van Esch National Institute of Public Health Utrecht, Netherlands	Mouse	SS
Mr. P. Derse, Dr. P. Nees W.A.R.F. Institute, Inc. Madison, Wisconsin	Rat	SS

* S = Saccharin (free acid)
SS = Sodium saccharin

Saccharin and its salts were removed from the generally recognized as safe (GRAS) list of substances when a new FDA (1972A) regulation was published. It is the first substance to be given an interim status under a novel category of the food additives regulations established by FDA (1972B). The regulatory status of saccharin prior to this point has been summarized (Zienty, 1971).

In the past, a few investigators concluded that saccharin was not entirely harmless. It, acting in the mouth, decreases gastric appetite secretion, in the stomach it increases gastric secretion and decreases peptic digestion, in the small intestine it decreases absorption; acting on erythrocytes it decreases hemolysis, these reactions cannot be explained by the osmotic factor. Saccharin in the blood, in proportion to its concentration, passes into the lymph, cerebrospinal fluid, saliva, tear, and mammary secretion. Some investigators reported that saccharin should not be used at all because of its depressant action upon the heart. In feeding dogs large amounts of saccharin over a period of 100 days, the only effect was diffused hyperemia in the kidneys, lungs, myocardium, and livers which suggests that it is not entirely innocuous (Suter, 1945).

The important aspect of the safety evaluation of saccharin is the detection, identification, and toxicological study of its metabolites. Excretion and metabolism of saccharin in man were studied by Byard, McChesney, Golberg, and Coulston (1974). The results of these studies were in accord with earlier observations in laboratory animals (Byard and Golberg, 1973; Kennedy et al., 1972; Matthews, Fields, and Fishbein, 1973; Minegishi et al. , 1972) and in man (McChesney and Golberg, 1973) in showing that more than 90 percent of saccharin was very rapidly excreted in the urine, in unchanged form. Faecal excretion, averaging

nearly 6 per cent of the dose was considerably greater than was anticipated from the value of approximately 1 per cent reported for that route in the monkey by Pitkin et al. (1971).

Fitzhugh et al. (1951) reported that saccharin increased incidence of the uncommon condition of abdominal lymphosarcoma in rats receiving diets supplemented with 5 per cent of saccharin. But the authors did not consider the increase was causally related to feeding of saccharin.

Allen et al. (1957) reported on a technique for the assessment of bladder carcinogenesis which employed the implantation of pellets containing test materials directly to the bladder lumen of the test mice. they reported a significant incidence of bladder tumors in mice receiving saccharin implants. The observation was confirmed by Bryan and Yoshida (1971) and also suggested that saccharin was a potential carcinogen. Studied by Price et al (1970) demonstrated that groups of rats fed saccharin/cyclamate mixtures developed bladder cancer. Other feeding studies with saccharin (Roe et al. , 1970) published at about the same time, demonstrated no carcinogenic effects.

Munro et al. (1975) reported the results of their studies that saccharin administration was not accompanied by an increase in tumor incidence, although high doses were associated with reduced body weight in both sexes and decreased longivity in male

rats. However, preliminary report of studies conducted at the Wisconsin Alumni Research Foundation (WARF) and the U.S. Food and Drug Administration (FDA) suggested that rats fed high doses of saccharin developed bladder tumors. In the WARF and FDA studies, saccharin was fed to the parent generation and continued for two years with the offsprings. Thus, the test animals were exposed to the saccharin in utero and presumably through the mother's milk.

The results of these two studies were shown in Table 3 which were statistically positive as determined by the committee. It will be noted that, in the per cent incidence column, the results are not highly significant; but they are significant enough to warrant concern as to the potential bladder tumor genicity of saccharin (Coon, 1975).

Table 3

Saccharin carcinogenesis test rats two years (F_0-F_1 Generation feeding; in utero exposure).
(After Coon, 1975)

Laboratory (Date Finished)	Saccharin in Diet (%)	No. Rats Started	No. Bladder tumor		Incidence (%)
			No. Rats	Sex	
WARF (1972)	0	20	0/10	M	0
	5	20	4/15	M	27
	0	20	0/10	F	0
	5	20	0/12	F	0
FDA (1973)	0	35	1/25	M	4
	7.5	35	7/23	M	32
	0	45	0/24	F	0
	7.5	45	2/31	F	7
	5	--	Negative	M,F	0

The reason why bladder cancer was observed in these two studies and not in others is a matter for speculation. Other possible differences in the test procedures included housing, diets, drinking water, duration of treatment, and the presence or absence of the bladder parasite *(Trichosomoides crassicauda)* which has been associated with bladder cancer in rodents (Chapman, 1969).

The presence of the bladder stones in the test animals which, under some conditions, will produce bladder cancer in rats (Weil et al., 1965), and the chemical purity of the saccharin used in the various tests also may have contributed to the differing results.

Stavric et al. (1974) have demonstrated that the major impurity in most commercial samples was OTS and that the concentration of OTS in saccharin varies not only from manufacturer to manufacturer but also from batch to batch within the same plant. Clayson (1974) studied about carcinogenic or co-carcinogenic properties of bladder calculi in rats.

Grice (1975) briefly outlined the animal studies with saccharin and OTS, in their laboratories. The hypothesis for the development of bladder cancer from OTS was that, OTS inhibits carbonic anhydrase in the kidneys, increasing the excretion of bicarbonate. This action could produce an alkaline urine,

favoring the production of stones in the kidneys and bladder. Irritation from stones over a long period of time could produce hyperplasia and finally tumors.

The debate about toxicity of saccharin in Canada results in the ban of its use as a sweetener in foods. The FDA, on the basis of studies sponsored by the Canadian Government said that it would ban the use of saccharin in all U.S. foods and drinks. The ban is put into effect under the so-called Delaney amendment 1958 to the Food and Drug Act which prohibits the use or sale any chemical "suspected" of causing cancer in man. The Canadian Health Protection Branch of the Department of Health and Welfare, after consultation with toxicologists in other countries, has now banned the saccharin from use in foods, cosmetics, and as a sweetening agent in drug preparations from July 1, 1977. The U.S. ban will come into effect after the FDA has allowed 60 days for public and corporate hearing (Washington (AP), 1977; Yudhanaraweesak, 1977).

Sweetness is a quality of food that is greatly preferred by most human. The origin of this preference is not known, although it may appear as early as the fourth fetal month (Bradley and Mistretta, 1972). Sweet preference is also evident in babies several days after birth. This preference is often maintained into adult life and has a number of deleterious effects, particularly dental caries and obesity. Sweet taste that satisfy the

taste sensation should be a pleasant sweetness without side tastes, should appear and disappear quickly as the stimulus is presented and withdrawn and develop nontoxic molecules that serve as sweet stimuli but do not appreciably affect the body's metabolism. Sucrose meets most of these requirements, but its caloric content is unacceptable for some one. Substitutes, such as saccharin, dulcin, and cyclamate do not have the pleasant sweet quality of sucrose and their toxicity may be questioned. Aspartylphenylalanine methyl ester (APM) has an excellent quality of sweetness but there remains the problem of amino acid imbalance. Miraculin (sweet principle of Miracle Fruit, *Synsepalum dulcificum*, Schum. et Thonn, Sapotaceae) has a good quality of sweetness. It is not toxic but can only be used for special purpose. Monellin (sweet tasting protein from *Dioscoreophyllum cumminsii*, Diels, Menispermaceae) quality is moderate but produces a lingering sweetness. To day there is a hurried search for more acceptable molecules (Beidler, 1974).

Because of high calories of these sweeteners, sucrose, glucose, fructose, the non-caloric synthetic sweeteners have a role in many food products. Saccharin has the sweetness about 300 times of sucrose, calcium cyclamate has 30 times as sweet as sucrose. Among artificial sweeteners, the most acceptable product is the combination of saccharin and cyclamate. But in 1969, cyclamate was banned in the United States.

There are several important criteria for a good synthetic sweetener that might be considered prerequisites for a new sweetening agent if it need to be successful commercially. Safety is the most important requirement. Several potential sweeteners have failed because of their toxicity, such as dulcin and P-4000. Now cyclamate and saccharin were banned in Canada. The natural exotic sweeteners such as stevioside and glycyrrhizin, have a history of use by various people. These natural products do not exhibit any sensational toxicity. New synthetic sweeteners are Aspartame (trade name of aspartylphenylalanine methyl ester) and natural non-sugar sweetener, dihydrochalcone, derived from citrus biflavonoid, especially neohesperidin dihydrochalcone (Beck, 1974).