SACCHARIN (Group 2B)

A. Evidence for carcinogenicity to humans (*inadequate*)

The evidence that the risk of cancer is increased among users of artificial sweeteners is inconsistent[1]. Since the positive report of Howe *et al.*[2], reports have become available on seven case-control studies and on one population study of bladder cancer.

The largest was a population-based study in ten areas of the USA, with 3010 bladder cancer cases and 5783 controls. The relative risk for bladder cancer associated with use of artificial sweeteners was 1.0 (95% confidence interval, 0.9-1.1) among men and 1.1 (0.9-1.3) among women. Significant trends of increasing risk with increasing average daily consumption were found in certain subgroups examined *a priori* on the basis of the results of animal experiments; these subgroups were female nonsmokers and male heavy smokers[3]. Subsequent, independent re-analysis of the same data by a different statistical technique (multiple logistic regression) confirmed the original findings overall but cast doubt on the significance of the findings in the two subgroups because of inconsistent dose-response trends, especially among the male heavy smokers[4]. In response, the original investigators noted that the inconsistency derived from the development of risk scores which, in their opinion, were not correctly derived, as two relevant variables had been omitted[5]. In a subsequent report on data from one of the areas participating in this study, the use of hospital and population controls was compared. A higher proportion of hospital controls was found to have used artificial sweeteners than population controls[6]. This had been postulated earlier[2] as a possible reason for the negative findings of a hospital-based case-control study[7]. Bias resulting from use of prevalent rather than incident cases[8] has been suggested as a possible reason for the negative findings of another hospital-based case-control study[9].

Three other case-control studies have also shown increased risks among subgroups. In one, conducted simultaneously in Japan, the UK and the USA, the relative risks among women in the US component of the study associated with 'any' use of diet drinks and of sugar substitutes were 1.6 and 1.5, respectively, and 2.6 and 2.1, respectively, for nonsmokers[10]. In the other two areas, however, a history of the use of sugar substitutes, primarily saccharin, was not associated with an elevated bladder cancer risk[11]. In a second study, conducted in West Yorkshire, UK, elevated risks were found for saccharin takers who were nonsmokers. In men, the relative risk was 2.2 (95% confidence interval, 1.3-3.8); that in women was 1.6 (0.8-3.2)[12]. In a third study, conducted in a rural district of Denmark, a relative risk of 2.5 (1.0-6.6) was reported for saccharin consumption in men and women combined. This risk was not reduced after controlling for tobacco use and industrial work[13].

Two studies in Denmark[14,15], one in the USA[16] and a further case-control study in Canada[17], however, gave negative results. In one of the Danish studies, incidence of bladder cancer at ages 20-34 among people born 1941-1945 (when use of saccharin was high in Denmark) was compared with that among those born 1931-1940. The risk for men was 1.0 (0.7-1.6) and that for women, 0.3 (0.1-1.0). This study indirectly assessed intrauterine exposure to saccharin[14]. The other two studies were population-based case-control studies of bladder cancer. In Denmark, the relative risk for people of each sex combined was 0.8 (0.6-1.1)[15]. In a study in the USA of bladder cancer in women aged 20-49, the odds ratio for regular use of artificially sweetened beverages, table-top sweetener or both was 1.1 (0.7-1.7)[16]. In Canada, the odds ratio for use of saccharin was 1.0 (0.9-1.2) in men and 1.0 (0.8-1.2) in women[17]. The increased risks seen in subgroups in other studies were not replicated in either study.

In the USA, in a study of 1862 patients hospitalized for cancer and of 10 874 control patients, a greater proportion of artificial sweetener users was found only among women with cancer of the stomach. Little information was available on urinary-tract cancer. No overall association was found between artificial sweetener use and cancer[18].

B. Evidence for carcinogenicity to animals (*sufficient*)

Saccharin (unspecified or commercial) has been tested for carcinogenicity by oral administration to mice, rats and hamsters. In mice, saccharin produced no difference in tumour incidence between treated and control animals in one single- and in one multi-generation study. Two further studies by oral administration in mice and three in rats were considered to be inadequate for evaluation. A study in hamsters by oral administration and one study in mice by skin application could not be evaluated. A study in mice by bladder insertion provided evidence for the induction of bladder carcinomas[1]. Oral administration to mice produced thyroid tumours[19].

Sodium saccharin has been tested for carcinogenicity by oral administration to mice, rats and monkeys. One study in mice was inadequate for evaluation[1]. One single-generation study in rats showed an increased incidence of bladder tumours in males; two further studies showed a few bladder tumours; another study showed no difference in tumour incidence between treated and control animals; and two others were inadequate for evaluation[1]. In four two-generation studies in rats, sodium saccharin produced a statistically significant

increase in the incidence of bladder tumours in F_1 males fed either 5% or 7.5% sodium saccharin[1,20]. In a further two-generation study of rats, a dose-related increase in the incidences of benign, malignant and/or combined bladder neoplasms was observed in males treated with doses ranging from 4-7.5% in the diet, while no tumorigenic effect was observed with 1%[21,22]. Transplacental exposure of rats to sodium saccharin and to saccharin (commercial) did not produce any treatment-related neoplasm[21,23]. Sodium saccharin has also been tested in mice by bladder insertion: it increased the incidence of bladder carcinomas. Experiments in which it was tested by oral administration to monkeys and by intraperitoneal administration to mice were considered to be inadequate for evaluation[1].

The combination of sodium saccharin with sodium cyclamate in a ratio of 1:10 has been tested by oral administration in a multigeneration experiment in mice and in single experiments in rats. In one study in rats, transitional-cell carcinomas in the bladder were produced in male animals given the highest dose; in two further studies in rats and in the study in mice, there was no difference in tumour incidence between treated and control animals[1,24]. Another study in rats was inadequate for evaluation[1].

Pretreatment with a single instillation into the bladder of a low dose of *N*-methyl-*N*-nitrosourea or feeding of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and subsequent oral administration of sodium saccharin for long periods increased the incidence of bladder neoplasms in rats over that induced by the nitrosourea or the amide alone[1]. Simultaneous administration of *N*-nitroso-*N*-(4-hydroxybutyl)butylamine and sodium saccharin significantly enhanced the induction of bladder papillomas over that seen after treatment with the nitrosamine alone[25]. Commercial saccharin preparations enhanced lung tumour induction in mice when given before or during intraperitoneal administration of urethane[26]. In rats, oral administration of sodium saccharin significantly increased the incidence of bladder neoplasms induced by ulceration of bladder mucosa[27,28]. Other studies of simultaneous or consecutive treatment with saccharin and known carcinogens were inadequate for evaluation[1].

ortho-Toluenesulphonamide was tested for carcinogenicity by oral administration in rats in a two-generation study: no increase in bladder tumour incidence was noted in animals of either generation. In one of two single-generation studies in rats, benign and malignant bladder tumours were found[1].

C. Other relevant data

No data were available on the genetic and related effects of saccharin, sodium saccharin or *ortho*-toluenesulphonamide in humans[29].

It should be noted that many studies do not differentiate between saccharin ('insoluble' form) and sodium saccharin. Additionally, when it is reported that 'saccharin' (presumably sodium saccharin) causes a positive response, primarily in assays for chromosomal effects, the effect is seen only with very high concentrations, at which simple salts also give responses[29].

Treatment of mice with saccharin did not induce micronuclei or chromosomal aberrations in bone-marrow cells or spermatocytes; conflicting results were obtained for the induction of dominant lethal mutations. A commercial preparation (of unknown purity) caused somatic mutations in the mouse spot test. Injection of radioactive saccharin into rats revealed no DNA binding in the liver or bladder, nor did treatment of rats result in DNA damage in bladder tissue. Saccharin did not induce sister chromatid exchanges in cultured human lymphocytes. Negative results were obtained in assays for transformation in cultured rodent cells, but saccharin enhanced transformation of virus-infected rat embryo cells and of C3H 10T1/2 mouse embryo cells initiated with 3-methylcholanthrene in two-state transformation assays. Results obtained with rodent cell systems were inconclusive with regard to inhibition of intercellular communication. It caused DNA strand breaks in rat hepatocytes but no chromosomal aberration in Chinese hamster cells. Saccharin induced aneuploidy but not recombination or gene conversion in yeast. It was not mutagenic and did not induce prophage in bacteria[29].

Treatment of mice with sodium saccharin did not induce micronuclei, somatic mutations (in the spot test) or sperm abnormalities. Treatment of Chinese hamsters did not induce chromosomal aberrations in bone-marrow cells or spermatogonia but induced sister chromatid exchanges in bone-marrow cells. Treatment of mice with commercial sodium saccharin resulted in the induction of dominant lethal mutations, but treatment with a preparation 'purified' by undefined criteria did not. Sodium saccharin induced chromosomal aberrations and sister chromatid exchanges in cultured human lymphocytes and induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells but no mutation in mouse lymphoma cells. It did not induce transformation of BALB/c 3T3 cells. Contradictory results have been reported concerning the ability of sodium saccharin to induce sex-linked recessive lethal mutations in *Drosophila*, and it did not cause a significant increase in heritable translocations. Sodium saccharin induced mutation, gene conversion and recombination in yeast, but was not mutagenic to bacteria[29].

ortho-Toluenesulphonamide did not induce micronuclei or somatic mutation (in the spot test) in mice treated *in vivo*. Contradictory results have been obtained for the induction of sex-linked recessive lethal mutations in *Drosophila*. It was not mutagenic to bacteria[29].

References

[1]*IARC Monographs*, *22*, 55-109, 171-185, 1980

[2]Howe, G.R., Burch, J.D., Miller, A.B., Cook, G.M., Estève, J., Morrison, B., Gordon, P., Chambers, L.W., Fodor, G. & Winsor, G.M. (1980) Tobacco use, occupation, coffee, various nutrients and bladder cancer. *J. natl Cancer Inst.*, *64*, 701-713

[3]Hoover, R.M. & Strasser, P.H. (1980) Artificial sweeteners and human bladder cancer. Preliminary results. *Lancet*, *i*, 837-840

[4]Walker, A.M., Dreyer, N.A., Friedlander, E., Loughlin, J., Rothman, K.J. & Kohn, H.I. (1982) An independent analysis of the National Cancer Institute study on non-nutritive sweeteners and bladder cancer. *Am. J. public. Health*, *72*, 376-381

[5]Hoover, R. & Hartge, P. (1982) Non-nutritive sweeteners and bladder cancer. *Am. J. public Health, 72*, 382-383

[6]Silverman, D.T., Hoover, R.N. & Swanson, G.M. (1983) Artificial sweeteners and lower urinary tract cancer: hospital vs. population controls. *Am. J. Epidemiol., 117*, 326-334

[7]Wynder, E.L. & Stellman, S.D. (1980) Artificial sweetener use and bladder cancer: a case-control study. *Science, 207*, 1214-1216

[8]Goldsmith, D.F. (1982) Calculation of potential bias in the odds ratio illustrated by a study of saccharin use and bladder cancer. *Environ. Res., 27*, 298-306

[9]Kessler, I.I. & Clark, J.P. (1978) Saccharin, cyclamate, and human bladder cancer. No evidence of an association. *J. Am. med. Assoc., 240*, 349-355

[10]Morrison, A.S. & Buring, J.E. (1980) Artificial sweeteners and cancer of the lower urinary tract. *New Engl. J. Med., 302*, 537-541

[11]Morrison, A.S., Verhoek, W.G., Leck, I., Aoki, K., Ohno, Y. & Obata, K. (1982) Artificial sweeteners and bladder cancer in Manchester, UK, and Nagoya, Japan. *Br. J. Cancer, 45*, 332-336

[12]Cartwright, R.A., Adib, R., Glashan, R. & Gray, B.K. (1981) The epidemiology of bladder cancer in West Yorkshire. A preliminary report on non-occupational aetiologies. *Carcinogenesis, 2*, 343-347

[13]Mommsen, S., Aagaard, J. & Sell, A. (1983) An epidemiological study of bladder cancer in a predominantly rural district. *Scand. J. Urol. Nephrol., 17*, 307-312

[14]Jensen, O.M. & Kamby, C. (1982) Intra-uterine exposure to saccharin and risk of bladder cancer in man. *Int. J. Cancer, 29*, 507-509

[15]Møller-Jensen, O., Knudsen, J.B., Sørensen, B.L. & Clemmesen, J. (1983) Artificial sweeteners and absence of bladder cancer risk in Copenhagen. *Int. J. Cancer, 32*, 577-582

[16]Piper, J.M., Matanoski, G.M. & Tonascia, J. (1986) Bladder cancer in young women. *Am. J. Epidemiol., 123*, 1033-1042

[17]Risch, H.A., Burch, J.D., Miller, A.B., Hill, G.B., Steele, R. & Howe, G.R. (1987) Dietary factors and the incidence of cancer of the urinary bladder. *Am. J. Epidemiol.* (in press)

[18]Morrison, A.S. (1979) Use of artificial sweeteners by cancer patients. *J. natl Cancer Inst., 62*, 1397-1399

[19]Prasad, O. & Rai, G. (1986) Induction of papillary adenocarcinoma of thyroid in albino mice by saccharin feeding. *Indian J. exp. Biol., 24*, 197-199

[20]Taylor, J.M., Weinberger, M.A. & Friedman, L. (1980) Chronic toxicity and carcinogenicity to the urinary bladder of sodium saccharin in the in utero-exposed rat. *Toxicol. appl. Pharmacol., 54*, 57-75

[21]Schoenig, G.P., Goldenthal, E.I., Geil, R.G., Frith, C.H., Richter, W.R. & Carlborg, F.W. (1985) Evaluation of the dose response and in utero exposure to saccharin in the rat. *Food chem. Toxicol., 23*, 475-490

[22]Squire, R.A. (1985) Histopathological evaluation of rat urinary bladders from the IRDC two-generation bioassay of sodium saccharin. *Food chem. Toxicol., 23*, 491-497

[23]Schmähl, D. & Habs, M. (1980) Absence of a carcinogenic response to cyclamate and saccharin in Sprague-Dawley rats after transplacental application. *Arzneimittelforschung, 30*, 1905-1906

[24]Schmähl, D. & Habs, M. (1984) Investigations on the carcinogenicity of the artificial sweeteners sodium cyclamate and sodium saccharin in rats in a two-generation experiment. *Arzneimittel-forschung, 34*, 604-606

[25]Nakanishi, K., Hirose, M., Ogiso, T., Hasegawa, R., Arai, M. & Ito, N. (1980) Effects of sodium saccharin and caffeine on the urinary bladder of rats treated with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. *Gann, 71*, 490-500

[26]Theiss, J.C., Arnold, L.J. & Shimkin, M.B. (1980) Effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice. *Cancer Res., 40*, 4322-4324

[27]Cohen, S.M., Murasaki, G., Fukushima, S. & Greenfield, R.E. (1982) Effect of regenerative hyperplasia on the urinary bladder: carcinogenicity of sodium saccharin and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. *Cancer Res., 42*, 65-71

[28]Hasegawa, R., Greenfield, R.E., Murasaki, G., Suzuki, T. & Cohen, S.M. (1985) Initiation of urinary bladder carcinogenesis in rats by freeze ulceration with sodium saccharin promotion. *Cancer Res., 45*, 1469-1473

[29]*IARC Monographs, Suppl. 6*, 488-493, 520-522, 1987