CYCLAMATES (Group 3)

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 six 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 cases and 5783 controls. The relative risk for bladder cancer associated with use of artificial sweeteners was 1.0 (95% confidence interval, 0.89-1.1) among men and 1.1 (0.89-1.3) 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].

Two 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 non-smokers[10]. In the other two areas, however, a history of use of sugar substitutes, primarily saccharin, was not associated with an elevated bladder cancer risk[11]. In the other study, conducted in West Yorkshire, UK, although elevated risks were found for saccharin takers (see p. 334) who were nonsmokers, the risks associated with cyclamate use were not examined[12].

Two studies in Denmark[13,14], one in the USA[15] and a further case-control study in Canada[16], 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)[13]. The other two studies were population-based case-control studies of bladder cancer. In Denmark, the relative risk for people of the two sexes combined was 0.78 (0.58-1.05)[14]. 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)[15]. In Canada, the odds ratio for use of cyclamate was 1.09 (0.60-1.97) in males and 0.92 (0.63-1.36) in females[16]. In neither study were the increased risks seen in subgroups in other studies replicated.

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[17].

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

Sodium cyclamate was tested for carcinogenicity both alone and in combination with other chemicals in different animal species and by several routes of administration. Following its oral administration to two strains of mice, an increased incidence of lymphosarcomas was observed in female mice of one strain; a few bladder tumours were seen in rats exposed orally. Several other experiments in mice, rats, hamsters and monkeys were inadequate for evaluation. A 10:1 mixture of sodium cyclamate:sodium saccharin was given to mice in one multigeneration experiment and to rats in two single-generation

experiments: transitional-cell carcinomas were induced in the bladders of male rats of one strain given the highest dose[1]. In a similar two-generation experiment in rats, no treatment-related tumour was observed[18]. Instillation of low doses of *N*-methyl-*N*-nitrosourea into the bladder of rats fed sodium cyclamate for long periods resulted in a dose-related induction of transitional-cell neoplasms of the bladder. After subcutaneous injection of rats with sodium cyclamate, no tumour was observed at the site of injection, the only site for which tumour incidence was reported. A significant increase in the incidence of bladder carcinomas was observed in mice given bladder implants of pellets containing sodium cyclamate[1]. Transplacental application of cyclamate to rats did not produce an increase in tumour incidence at any site[19].

Calcium cyclamate did not alter tumour incidence when tested by oral administration in a two-generation experiment in rats but produced local tumours in another experiment following its subcutaneous injection[1].

Cyclohexylamine was tested by oral administration at several dose levels in different strains of mice and rats, and in one multigeneration study in mice. No tumour related to treatment was observed[1].

C. Other relevant data

No data were available on the genetic and related effects of calcium cyclamate, dicyclohexylamine or cyclohexylamine in humans. In a single study, eight persons ingesting sodium cyclamate (70 mg/kg per day) did not exhibit chromosomal aberrations in their lymphocytes[20].

Calcium cyclamate induced chromosomal aberrations in bone-marrow cells of gerbils, but not in bone-marrow cells or spermatogonia of rats, treated *in vivo*. It did not induce dominant lethal mutations in rats or mice or micronuclei or sperm abnormalities in mice treated *in vivo*. It induced chromosomal aberrations in human lymphocytes but not in rat kangaroo cells in culture. It did not induce aneuploidy in *Drosophila*, but contradictory results were reported in assays for sex-linked recessive lethal mutations and heritable translocations. Calcium cyclamate was not mutagenic to bacteria[20].

Sodium cyclamate did not induce dominant lethal mutations or chromosomal aberrations in spermatogonia or spermatocytes of mice treated *in vivo*. It induced sister chromatid exchanges and chromosomal aberrations in cultured human lymphocytes and chromosomal aberrations in cultured Chinese hamster cells. It did not induce aneuploidy or sex-linked recessive lethal mutations in *Drosophila* or chromosomal aberrations in plants[20].

Cyclohexylamine did not induce dominant lethal mutations in one study in rats, but contradictory results were obtained in mice. It gave weakly positive results in the mouse spot test. Cyclohexylamine induced chromosomal aberrations in lymphocytes but not in bone-marrow cells of hamsters and lambs or in spermatogonia of hamsters and mice treated *in vivo*. In treated rats, chromosomal aberrations were induced in spermatogonia but not in leucocytes, and contradictory results were obtained for bone-marrow cells. Cyclohexylamine induced sister chromatid exchanges in cultured human lymphocytes, but, again,

conflicting results were obtained concerning the induction of chromosomal aberrations. Cyclohexylamine enhanced virus-induced transformation of Syrian hamster embryo cells and induced chromosomal aberrations in cultured rat kangaroo cells. It did not induce somatic or sex-linked recessive lethal mutations, aneuploidy or heritable translocations in *Drosophila* and was not mutagenic and did not induce prophage in bacteria. In host-mediated assays, it did not induce mutation in bacteria or chromosomal aberrations in human leucocytes[20].

Dicyclohexylamine induced chromosomal aberrations in cultured human lymphocytes. It was not mutagenic to bacteria[20].

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