CYCLAMATES

These substances were considered by previous working groups, in 1979 (IARC, 1980) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Cyclamic acid

Chem. Abstr. Serv. Reg. No.: 100-88-9
Deleted CAS Reg. No.: 45951-45-9
Chem. Abstr. Name: Cyclohexylsulfamic acid
IUPAC Systematic Name: Cyclohexanesulfamic acid
Synonyms: Cyclamate; cyclohexylamidosulfuric acid; cyclohexylaminesulfonic acid; *N*-cyclohexylsulfamic acid; hexamic acid; sucaryl; sucaryl acid

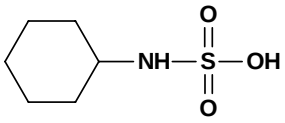
Sodium cyclamate

Chem. Abstr. Serv. Reg. No.: 139-05-9
Deleted CAS Reg. No.: 53170-91-5
Chem. Abstr. Name: Cyclohexylsulfamic acid, monosodium salt
IUPAC Systematic Name: Cyclohexanesulfamic acid, monosodium salt
Synonyms: Cyclamate sodium; cyclohexylsulfamate sodium; *N*-cyclohexylsulfamic acid sodium salt; sodium cyclohexanesulfamate; sodium cyclohexylaminesulfonate; sodium cyclohexylsulfamate; sodium sucaryl; sucaryl sodium

Calcium cyclamate

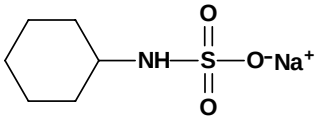
Chem. Abstr. Serv. Reg. No.: 139-06-0
Deleted CAS Reg. No.: 201280-50-4
Chem. Abstr. Name: Cyclohexylsulfamic acid, calcium salt (2:1)
IUPAC Systematic Name: Cyclohexanesulfamic acid, calcium salt (2:1)
Synonyms: Calcium cyclohexanesulfamate; calcium sucaryl

1.1.2 *Structural and molecular formulae and relative molecular mass*



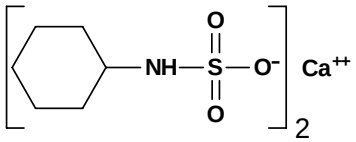
Cyclamic acid

$C_6H_{13}NO_3S$ Relative molecular mass: 179.2



Sodium cyclamate

$C_6H_{12}NO_3S.Na$ Relative molecular mass: 201.2



Calcium cyclamate

$(C_6H_{12}NO_3S)_2Ca$ Relative molecular mass: 396.5

1.1.3 *Chemical and physical properties of the pure substances*

From Lewis (1993) and Budavari (1996) unless otherwise noted

Cyclamic acid

(*a*) *Description*: White odourless, crystalline powder with a sweet taste
(*b*) *Melting-point*: 169–170°C
(*c*) *Solubility*: Soluble in water (130 g/L) and ethanol (Bopp & Price, 1991); insoluble in oils
(*c*) *pH of a 10% aqueous solution*: 0.8–1.6 (Bopp & Price, 1991)
(*d*) *Conversion factor*: $mg/m^3 = 7.33 \times ppm$

Sodium cyclamate

(*a*) *Description*: White, odourless, crystalline powder with a sweet taste (about 30 times as sweet as refined cane sugar)
(*b*) *Solubility*: Freely soluble in water; practically insoluble in benzene, chloroform, ethanol and diethyl ether
(*c*) *pH of a 10% aqueous solution*: 5.5–7.5
(*d*) *Conversion factor*: $mg/m^3 = 8.23 \times ppm$
(*e*) Stability: Cyclamate solutions are stable to heat, light and air throughout a wide range of pH.

Calcium cyclamate

(*a*) *Description*: White odourless, crystalline powder with a sweet taste, somewhat less than that of sodium cyclamate
(*b*) *Solubility*: Freely soluble in water; practically insoluble in benzene, chloroform ethanol and diethyl ether
(*c*) *pH of a 10% aqueous solution*: Neutral to litmus (5.5–7.5)
(*d*) *Conversion factor*: $mg/m^3 = 16.22 \times ppm$
(*e*) *Stability*: More resistant to cooking temperatures than saccharin

1.2 Production and use

Cyclamates are produced from cyclohexylamine (obtained by the reduction of aniline) by sulfonation (Bizzari *et al*., 1996).

Production of sodium and calcium cyclamates in western Europe (annual capacity, 4200 tonnes) in 1995 was estimated to be 4000 tonnes. Brazil (annual capacities of 4000 and 2000 tonnes of sodium and calcium cyclamate, respectively) produced over 2300 and 300 tonnes of sodium and calcium cyclamate, respectively, in 1993. There is no commercial production of cyclamates in Japan or the United States (Bizzari *et al*., 1996).

Information available in 1995 indicated that cyclamic acid was produced in Japan, Spain, Taiwan and the United States, that sodium cyclamate was produced in Argentina, Brazil, Germany, Indonesia, Japan, the Netherlands, Romania, South Africa, Spain, Taiwan, Thailand and the United States and that calcium cyclamate was produced in Argentina, Brazil, Japan, South Africa, Taiwan and the United States (Chemical Information Services, 1995).

Sodium and calcium cyclamates are used as non-nutritive sweeteners (Lewis, 1993; Budavari, 1996); sodium cyclamate, simply known as ‘cyclamate’, is the more common salt. Calcium cyclamate is used in low-sodium and sodium-free products. World consumption of cyclamates in 1995 was estimated to be 15 000 tonnes. Western European consumption of cyclamates was estimated to be 4000 tonnes. Canadian consumption of cyclamates in 1995 was estimated to be 60 tonnes. Consumption of sodium cyclamate in China in 1995 was estimated to be 4400 tonnes (Bizzari *et al*., 1996).

1.3 Occurrence

1.3.1 *Natural occurrence*

Cyclamates are not known to occur naturally.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 5300 workers in the United States were potentially exposed to sodium cyclamate. Occupational exposure to sodium cyclamate may occur during its production.

1.3.3 *Dietary intake*

The dietary intake of cyclamate in Spain was evaluated in 1992. The average daily intake of cyclamate was 0.44 mg/kg bw for the whole population and 2.44 mg/kg bw among consumers of cyclamates. Subjects following a diet, such as diabetic patients, reported the highest intakes, and only 0.16% of the sample studied consumed more than the acceptable daily intake (ADI) of 11 mg/kg bw (Serra-Majem *et al*., 1996).

A survey of intense sweetener consumption in Australia was conducted in 1994, which consisted of a seven-day survey of high consumers of the main sources of sweeteners (i.e. carbonated drinks, cordials and table-top sweeteners), with allowance for body weight. The mean intake (expressed as percentage of the ADI of 11 mg/kg bw) of cyclamates was 23% by all consumers 12–39 years of age (men, 27%; women, 21%), 34% by all consumers 12–17 years of age, 20% by all consumers 18–24 years of age and 20% by all consumers 25–39 years of age (National Food Authority, 1995).

In a study of the potential intake of intense sweeteners in Brazil in 1990–91, 67% of the studied population was found to consume cyclamate. Table-top sweeteners were the major source, followed by soft drinks. The median daily intake of cyclamate was approximately 16% of the ADI (11 mg/kg bw) (Toledo & Ioshi, 1995).

The use of table-top sweeteners and diet soft-drinks and intake of cyclamate were assessed from the second Dutch National Food Consumption Survey, conducted in 1992. Users of intense sweeteners assessed from two-day records had a median daily intake of cyclamate of 0.8 mg/kg bw; those assessed from a food frequency questionnaire had a median daily intake of 1.1 mg/kg bw. Less than 0.5% of the total population had an intake above the ADI (11 mg/kg bw) (Hulshof *et al*., 1995).

The dietary intake of intense sweeteners was evaluated in Germany in 1988–89. Complete 24-h records of the amounts and types of all foods and drinks consumed were obtained from 2291 individuals. The mean intake of cyclamate by users of intense sweeteners was 2.62 mg/kg bw per day. Sweetener intake was further evaluated during a seven-day period in those subjects who in the one-day study had a consumption of any of the sweeteners in excess of 75% of the ADI (11 mg/kg bw). The mean intake of cyclamate for this group was 4.53 mg/kg bw per day, corresponding to 41% of the ADI (Bär & Biermann, 1992).

A survey of general food additive intakes in Finland in 1980 included an assessment of the intake of cyclamate (Penttilä *et al*., 1988). The paper gave few details of the study design or method and reported only that the average daily intake of sodium cyclamate (calculated from data on the consumption of various foods and drinks) was 12.3 mg/person.

1.4 Regulations and guidelines

No national or international occupational exposure limits have been proposed for cyclamic acid, sodium cyclamate, or calcium cyclamate in workplace air, and no international guidelines for cyclamates in drinking-water have been established (WHO, 1993).

Cyclamate is approved for use in over 55 countries, including Canada (for table-top use only), Australia, and the European Union (Bizzari *et al*., 1996). An ADI of 11 mg/kg bw

for cyclamate was established by the WHO/FAO Joint Expert Committee on Food Additives and the Scientific Committee for Food of the European Union (Renwick, 1995). Cyclamate was banned in the United States in 1970 (Bopp & Price, 1991).

2. Studies of Cancer in Humans

Studies on artificial sweeteners in which the actual compound used was not specified are summarized in the monograph on saccharin; however, four of the studies included specific analyses of the risk for urinary bladder cancer associated with exposure to cyclamates (Simon *et al*., 1975; Kessler & Clark, 1978; Møller Jensen *et al*., 1983; Risch *et al*., 1988).

In the study of Simon *et al*. (1975; see the monograph on saccharin), cyclamate use was evaluated in coffee-drinkers and tea-drinkers separately. Among the coffee-drinkers (122 cases and 349 controls), nine patients with bladder cancer and 22 controls reported use of cyclamate, yielding a crude odds ratio of 1.2 (95% confidence interval [CI], 0.5–2.6). Among the tea-drinkers (98 cases and 281 controls), eight patients and 20 controls reported use of cyclamate, yielding a crude odds ratio of 1.2 (95% CI, 0.5–2.7). The groups of tea-drinkers and coffee-drinkers were mutually exclusive.

In the study of Kessler and Clark (1978), 130 of the 519 patients (25%) and 135 of the 519 controls (26%) had ever used cyclamate, yielding an odds ratio of 1.0 (95% CI, 0.7–1.3). The risk did not differ substantially between men (odds ratio, 1.1; 95% CI, 0.8–1.6) and women (odds ratio, 0.7; 95% CI, 0.5–1.2), and the results did not change when the odds ratios were adjusted in a multivariate analysis for several potential confounders (smoking, occupation, age, race, sex, diabetes mellitus, marital status, education, overweight, dieting and memory) or when the odds ratios for different types of artificial sweeteners were estimated simultaneously.

In the study of Risch *et al*. (1988), odds ratios of 1.1 (95% CI, 0.60–2.0) for men and 0.9 (95% CI, 0.6–1.4) for women were reported when 'total lifetime intake' was included in a continuous regression model and after adjustment for lifetime cigarette consumption and history of diabetes, but not for other use of artificial sweeteners.

In the study of Møller Jensen *et al*. (1983), 11% of the subjects reported having used cyclamate only. There was no indication that cyclamate users had an elevated risk. The odds ratio was 0.72 (95% CI, 0.3–2.0) for men and 1.3 (95% CI, 0.22–8.3) for women.

3. Studies of Cancer in Experimental Animals

Previous evaluation

Sodium cyclamate was tested by oral administration in two experiments in mice, one of which was a multigeneration study, and in three experiments in rats. A few benign and malignant bladder tumours were observed in rats, but the incidences were not statistically

greater than those in controls in any single experiment. An increased incidence of lymphosarcomas was seen in female but not in male mice in one experiment. Sodium cyclamate was also tested by oral administration in other experiments in mice, rats, hamsters and monkeys, but these experiments could not be evaluated because of various inadequacies or incomplete reporting.

Pellets of sodium cyclamate in cholesterol have been tested in mice by implantation into the bladder in one experiment: exposure by this route increased the incidence of bladder carcinomas.

When sodium cyclamate was administered to rats in one experiment by subcutaneous injection, no tumours were seen at the site of injection.

Calcium cyclamate has been tested by oral administration in one two-generation experiment in rats; no difference in tumour incidence was seen between treated and control animals. Two further experiments in rats showing a few bladder tumours and one in hamsters were considered to be inadequate for evaluation. When calcium cyclamate was administered to rats by subcutaneous injection, tumours were produced at the site of injection.

In one study in rats fed sodium cyclamate after receiving a single instillation into the bladder of a low dose of *N*-nitroso-*N*-methylurea, transitional-cell neoplasms of the bladder were produced. No such tumours were observed in animals that received *N*-nitroso-*N*-methylurea alone. [The present Working Group noted inadequacies in this study and noted that these results were not repeated in a second study.]

Cyclohexylamine, an intestinal biotransformation product of cyclamates, has been tested by oral administration in two experiments in mice, one of which was a multi-generation study, and in four experiments in rats; there were no differences in tumour incidence between treated and control animals. A further experiment in rats was considered to be inadequate for evaluation (IARC, 1980).

[The present Working Group noted, in relation to the study of Bryan and Ertürk (1970) summarized in the previous monograph, that it is difficult to interpret the results of studies involving bladder implantation, since the pellet itself has significant carcinogenic effects (Clayson, 1974; Jull, 1979; De Sesso, 1989). Furthermore, virtually all of the sodium cyclamate was reported to have leached out of the pellets within 7 h of implantation (Bryan & Ertürk, 1970), which would have left the pellet with a roughened surface, unlike the control pellets (De Sesso, 1989). The low incidence of bladder tumours in the groups receiving control pellets in the study of Bryan and Ertürk, which was not seen in subsequent studies involving implantation of pellets in the bladder, was also noted. The Working Group also noted, in relation to the study of Brantom *et al*. (1973) in which an increased incidence of lymphosarcomas was reported in female mice, that the study included only limited sampling of tissues for histopathological examination.]

For summaries of studies of combined administration of saccharin and cyclamates, see the monograph on saccharin and its salts.

New studies

No new data were available to the Working Group.

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Absorption of cyclamate from the gut is incomplete, and absorbed cyclamate is excreted in the urine. When three men received 1 g calcium [^{14}C]cyclamate orally, 87–90% was recovered in the urine and faeces in about equal amounts within four days (Renwick & Williams, 1972a). When the subjects had received a cyclamate-containing diet for 17–30 days before the radioactive dose, 96–99% of the dose was recovered within four to five days, and urinary excretion was greater than before the cyclamate diet. Most humans convert only small amounts of cyclamate to cyclohexylamine, and the majority converted < 0.1–8%; however, there is wide interindividual variation in the daily urinary excretion of cyclohexylamine, which can amount to 60% of a dose of cyclamate (IARC, 1980; Buss *et al*., 1992). Gastrointestinal microflora are the source of the conversion of unabsorbed cyclamate to cyclohexylamine (Drasar *et al*., 1972). Other aspects of the conversion of cyclamate to cyclohexylamine have been reviewed extensively (National Research Council, 1985; Ahmed & Thomas, 1992).

Two metabolites were definitely identified in the urine of volunteers who received an oral dose of [^{14}C]cyclohexylamine, namely cyclohexanol and *trans*-cyclohexane-1,2-diol. No *N*-hydroxycyclohexylamine was found in human urine (Renwick & Williams, 1972b).

4.1.2 *Experimental systems*

In guinea-pigs, rats and rabbits, 30, 50 and 5% of orally administered cyclamate was excreted in the faeces and 65, 40 and 95% in the urine, respectively, over two to three days. Cyclamate thus appears to be readily absorbed by rabbits but less readily by guinea-pigs and rats (Renwick & Williams, 1972a).

When given to lactating dogs and rats, calcium cyclamate reached higher concentrations in the milk than in the blood (Sonders & Wiegand, 1968; Ward & Zeman, 1971). In pregnant rats, significant amounts of sodium cyclamate were distributed to fetal tissues (Schechter & Roth, 1971). In rhesus monkeys, cyclamate crossed the placenta (Pitkin *et al*., 1969).

Cyclohexylamine can be formed to a variable extent by microbial biotransformation of cyclamate in the gastrointestinal tract of all species studied; after absorption, it is further metabolized to several compounds that are excreted in the urine (Golberg *et al*., 1969; Parekh *et al*., 1970; Asahina *et al*., 1971; Ichibagase *et al*., 1972; Coulston *et al*., 1977).

Administration to rats of 0.1% calcium cyclamate in the diet for eight months, followed by a single dose of [^{14}C]cyclamate, resulted in traces of dicyclohexylamine in the urine, but no *N*-hydroxycyclohexylamine was detected. Rats that had received a control diet did not convert [^{14}C]cyclamate to additional products (Prosky & O'Dell, 1971).

Most of the cyclohexylamine given by gavage or intraperitoneal injection to rats and guinea-pigs was excreted unchanged, and only 4–5% was metabolized within 24 h. In rabbits, 30% was metabolized. Cyclohexylamine has been reported to be metabolized further to cyclohexanone and then to cyclohexanol in guinea-pigs, rabbits and rats. A number of hydroxylated products of cyclohexylamine have been reported in these species, which were excreted in part as glucuronides (Renwick & Williams, 1972b).

In rhesus monkeys receiving 200 mg/kg bw cyclamate orally for several years, more than 99.5% was excreted unchanged. The principal metabolites found were cyclohexylamine, cyclohexanone and cyclohexanol (Coulston *et al*., 1977).

Comparison of the integrated area under the concentration–time curve (AUC) in the testis of rats and mice showed that the lower clearance of cyclohexylamine in rats resulted in greater exposure of the target organ for toxicity. In rat plasma and testes, the cyclohexylamine concentration was non-linearly related to intake, and elevated concentrations were found in these tissues after administration of doses above 200 mg/kg per day because of decreased urinary output. At a dose of 400 mg/kg per day, which caused testicular atrophy in rats but not mice, the plasma and testicular levels of cyclohexylamine were lower in mice than in rats (Roberts & Renwick, 1989; Roberts *et al*., 1989).

4.1.3 *Comparison of humans and rodents*

Orally administered cyclamate appears to be readily absorbed by rabbits but less readily by guinea-pigs, rats and humans. All of these species convert cyclamate to cyclohexylamine, *via* the action of gastrointestinal microflora on unabsorbed cyclamate. The metabolism of cyclohexylamine to other products differs somewhat in humans and other species, although most cyclohexylamine is rapidly excreted unchanged in the urine. In rats, it is metabolized mainly by hydroxylation of the cyclohexane ring; in humans, it is metabolized by deamination; and in guinea-pigs and rabbits, it is metabolized by ring hydroxylation and deamination (Renwick & Williams, 1972b).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The oral LD_{50} of sodium cyclamate in mice and rats is 10–12 g/kg bw (Richards *et al*., 1951); the oral LD_{50} in male and female hamsters—based on eight days' administration in drinking-water and calculated on the basis of mortality up to day 16—was 9.8

and 12 g/kg bw, respectively; for calcium cyclamate, the respective values were 4.5 and 6 g/kg bw (Althoff *et al*., 1975).

Several studies have shown that cyclohexylamine affects the testis of rats (Oser *et al*., 1976; Mason & Thompson, 1977). In one study, cyclohexylamine produced testicular atrophy in DA and Wistar rats, but not in mice, at a dietary dose of 400 mg/kg per day for up to 13 weeks. The testicular toxicity was not due to the formation of hydroxylated metabolites but to cyclohexylamine *per se*. The lack of sensitivity of mouse testis is probably due to the lower tissue concentrations of cyclohexylamine in this species in comparison with rats (Roberts *et al*., 1989).

Sodium cyclamate inhibited binding of ^{125}I-labelled mouse epidermal growth factor to dog kidney, rat hepatoma and rat liver cells, mouse fibroblasts, human foreskin and human skin fibroblasts, human carcinoma and human xeroderma pigmentosum cells *in vitro* (Lee, 1981).

In cultured bladders from young female Fischer 344 rats, 12 or 24 mmol/L sodium cyclamate produced pronounced urethelial hyperplasia and dysplasia, as confirmed by histology (Knowles *et al*., 1986). Sodium cyclamate alone or after exposure to *N*-methyl-*N*-nitrosourea induced foci of cell proliferation in explanted bladder epithelium cultures derived from young female Fischer 344 rats (Knowles & Jani, 1986; Nicholson & Jani, 1988).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

No adverse effects on fetal viability, growth or morphology as a consequence of exposure to cyclamates were reported in mice, rats, rabbits, hamsters, dogs and rhesus monkeys exposed during some or all periods of prenatal development (Fritz & Hess, 1968; Klotzsche, 1969; Lorke, 1969; Adkins *et al*., 1972). The chemical form used was usually the sodium salt, which was given either orally or in the diet. The concentrations used in the studies in rodents were up to 5% in the diet; in the study in dogs (Fancher *et al*., 1968), doses of up to 1 g/kg of a 10:1 combination of sodium cyclamate:saccharin were used; and monkeys received up to 2000 mg/kg on one of several four-day periods during gestation (Wilson, 1972). Adverse effects attributed to cyclamates were noted in two other studies. In one, reduced postnatal growth and altered reproductive capacity were found in the offspring of rats given diets containing 5 or 10% calcium cyclamate; food intake was markedly reduced in both treatment groups (Nees & Derse, 1965, 1967). In the other study, offspring of rats treated with 300 mg/L sodium and calcium cyclamate in the drinking-water during pregnancy had increased motor activity (Stone *et al*., 1969a).

In their review of the toxicity of cyclamates, Bopp *et al*. (1986) summarized a number of studies on the effects of these compounds on the testis, reproductive function and prenatal development, some of which were unpublished. With regard to male reproductive

effects, they concluded that the testicular atrophy observed in cyclamate-treated rats was associated with exposure to dietary concentrations of 5–10% (i.e. about 2.5–5.0 g/kg bw per day), was accompanied by reductions in body weight and was evident only after long-term exposure and hence appeared to be a secondary or indirect effect of exposure. [The Working Group noted that testicular effects are generally not seen in dietary restriction studies until body-weight deficits of more than 80% occur (Chapin & Gulati, 1997).] The authors also noted that the testicular effects were present only in rats (and not mice, dogs or rhesus monkeys). With regard to effects on reproductive function, they concluded that high doses adversely affected the viability and growth of rat pups during lactation, although the pups might also have eaten their mothers' feed, and that mice and dogs are insensitive to such exposure. With regard to developmental toxicity, they noted that numerous investigations in mice, rats and rabbits and isolated studies in dogs and rhesus monkeys have shown no reproducible effect of cyclamate on embryonic viability or on the frequency of malformations after exposure during organogenesis.

Sodium cyclamate did not affect growth or viability or induce malformations in a short-term screening test for teratogenicity at a minimally maternally toxic dose given orally on days 8–12 of gestation to ICR/SIM mice (Seidenberg *et al*., 1986).

Cyclamate did not affect the development of cultured rat embryos exposed on days 9.5–11.5 of gestation to concentrations of up to 810 μg/mL (Cicurel & Schmid, 1988). As reported in an abstract, exposure of NMRI mouse embryos to sodium cyclamate [dose not given] in culture for 26 h beginning on day 8.5 of gestation was not teratogenic (Delhaise *et al*., 1989).

The concentration of cyclamic acid that caused 50% inhibition of proliferation of human epithelial palatal mesenchyme cells was reported to be 6500 μg/mL (32 mmol/L). The authors considered that a concentration of less than 1 mmol/L was indicative of teratogenic potential in this assay (Pratt & Willis, 1985). A subsequent interlaboratory comparison provided no evidence that sodium cyclamate is teratogenic in either this cell growth inhibition assay or the mouse ovarian tumour cell attachment inhibition assay, which monitors cell attachment to concanavalin A-coated beads (Steele *et al*., 1988).

Cyclamate was considered to be ineffective in a test with rat embryonic limb bud micromass in culture. The mean concentrations that affected chondrogenesis and cell proliferation were 6200 and 3100 μg/mL, respectively (Renault *et al*., 1989).

Exposure of developing *Drosophila* larvae to cyclamate caused a dose-related increase in the number of extra bristles and reduced the whole-body size of adults, but these effects were not considered to be the most predictive of developmental toxicity in mammals (Lynch *et al*., 1991).

The effects of sodium cyclamate on the development of frog embryos were evaluated in a validation study in seven laboratories. The median lethal concentration, the concentration that induced malformations in 50% of the surviving embryos and the teratogenic index, i.e. the ratio of the two other measures, were 16 mg/mL, 14 mg/mL and 1.1 mg/mL, respectively. These results suggest that sodium cyclamate has low potential selective embryotoxicity (Bantle *et al*., 1994).

Cyclohexylamine

Studies have been carried out in rats, mice and rhesus monkeys exposed to cyclohexylamine during some or all of gestation and in a one-generation study in mice and a multigeneration study in rats. The exposure included intraperitoneal injection on a single day of gestation (mice; Gibson & Becker, 1971), oral administration for several days during pregnancy (mice, Takano & Suzuki, 1971; rats, Tanaka *et al*., 1973; and monkeys, Wilson, 1972) and long-term dietary administration (Lorke & Machemer, 1975; Kroes *et al*., 1977). No treatment-related malformations were seen in these studies, but several found evidence of developmental toxicity in the form of reduced growth or viability of fetuses and/or postnatal offspring (see also IARC, 1980).

Exposure of day-10.5 rat embryos in whole-embryo cultures to 1.0 mmol/L cyclohexylamine reduced growth and increased the incidence of morphologically abnormal embryos. No significant effects were seen at 0.3 mmol/L, and the addition of hepatic microsomes did not alter the dose–response relationship (Kitchin & Ebron, 1983).

In their review of the toxicity of cyclohexylamine, Bopp *et al*. (1986) summarized a number of studies of effects on the testis, reproductive function and prenatal development, some of which were unpublished. They concluded that the testicular effects, which included reduced organ weights, sperm counts, sperm motility and impairment of spermatogenesis, were among the most sensitive effects of cyclohexylamine at the target organ and that rats were more sensitive than other species, but that the mode of action was not discernible; hyperthermia, direct endocrine imbalance and primary Sertoli cell toxicity were dismissed as possible explanations for the testicular effects. With regard to effects on reproductive function, they noted that exposed male rats showed reduced fertility (evidenced by fewer impregnated females and implantation sites) and that mice had reduced litter sizes and pup weights at birth. In studies of prenatal toxicity, no malformations were seen after exposure of mice, rats, rabbits or monkeys to cyclohexylamine during gestation, but a study in mice and another in rats showed potential embryolethality. One study in which rat embryos were exposed *in vitro* showed an effect on growth and morphogenesis from doses of 1 mmol/L (Kitchin & Ebron, 1983). None of the studies of mammals *in vivo* demonstrated teratogenic effects.

4.4 Genetic and related effects

The genetic toxicology and other toxicological aspects of cyclamate and its principal metabolite cyclohexylamine have been reviewed (Bopp *et al*., 1986).

4.4.1 *Humans*

Chromosomal breaks were observed in lymphocytes from 11 human patients suffering from chronic liver and kidney diseases, who had taken daily doses of cyclamate (2–5 g) for up to three years. The number of chromosomal breaks per cell in this group was significantly higher than that in a similar group of patients who were not taking cyclamate or in healthy controls. The frequency of other types of cytogenetic damage, however, was significantly different only between the two patient groups and the controls

(Bauchinger *et al*., 1970). The other study involved three groups of healthy volunteers, each comprising two men and two women 20–54 years of age. The first group received no cyclamate and served as the control group. The second group consisted of four subjects who were capable of metabolically converting cyclamate to cyclohexylamine, and the third group comprised four subjects who could not convert cyclamate to cyclohexylamine. The last two groups received sodium cyclamate capsules three times a day for four days, providing an average daily dose of 70 mg/kg bw. Analysis of peripheral blood lymphocytes collected on the first and fifth days of the study showed no increase in the frequency of chromosomal aberrations in the two treated groups over that in the control subjects (Dick *et al*., 1974). [The Working Group considered that the second study was rather weak because of the limited size of the treated groups and the short exposure.]

4.4.2 *Experimental systems* (see Tables 1–3 for references)

Calcium cyclamate

In one study, calcium cyclamate did not induce gene mutation in *Salmonella typhimurium* strains TA100, TA1537 or TA98 in the presence or absence of exogenous metabolic activation at the highest concentration tested. It did not induce aneuploidy in *Drosophila melanogaster*; but in one of three studies it induced sex-linked recessive lethal mutations, and in one of two studies it induced heritable translocations when males were exposed to calcium cyclamate in the diet.

In separate studies, calcium cyclamate did not induce unscheduled DNA synthesis in rat primary hepatocyte cultures, gene mutation in Chinese hamster ovary cells at the *hprt* locus with or without exogenous metabolic activation, or chromosomal aberrations in a rat-kangaroo kidney cell line. Calcium cyclamate induced chromosomal aberrations in human lymphocytes cultured *in vitro* in the absence of metabolic activation.

Calcium cyclamate did not affect sperm morphology or induce micronuclei in the bone marrow of mice treated on five consecutive days by intraperitoneal injection, bone marrow or sperm samples being collected 4 h or 35 days, respectively, after the last injection. Chromosomal aberrations were induced in the bone marrow of Mongolian gerbils given five daily intraperitoneal injections of calcium cyclamate. Aberrations were not induced in the bone marrow or spermatogonia of rats given calcium cyclamate and casein in a semisynthetic diet for 10 months, nor were dominant lethal mutations observed in these rats. Calcium cyclamate did not induce dominant lethal mutations in mice of two strains given calcium cyclamate by gavage daily for five or 30 days, respectively. In a single study, calcium cyclamate did not induce heritable translocations in mice given the compound by gavage on five days per week for six weeks.

Sodium cyclamate

In one study, the frequency of chromosomal aberrations in the callus and root tips of *Haworthia* exposed to sodium cyclamate *in vitro* was not significantly greater than that in control cultures. Sodium cyclamate did not induce sex-linked recessive lethal mutation or aneuploidy in *D. melanogaster*.

Table 1. Genetic and related effects of calcium cyclamate

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Salmonella typhimurium TA100, TA98, TA1537, reverse mutation	–	–	500 µg/plate	Bruce & Heddle (1979)
Drosophila melanogaster, sex-linked mutation	+		50000 ppm feed	Majumdar & Freedman (1971) [abst]
Drosophila melanogaster, sex-linked mutation	–		50000 ppm feed	Rotter & Mittler (1972) [abst]
Drosophila melanogaster, sex-linked mutation	–		100 mg/mL feed	Brusick *et al*. (1989)
Drosophila melanogaster, heritable translocations	–		50000 ppm feed	Rotter & Mittler (1972) [abst]
Drosophila melanogaster, heritable translocations	+		12500 ppm feed	Wu & Smith (1981) [abst]
Drosophila melanogaster, aneuploidy	–		50000 ppm feed	Rotter & Mittler (1972) [abst]
Drosophila melanogaster, aneuploidy	–		12500 ppm feed	Wu & Smith (1981) [abst]
Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	–	NT	5000	Brusick *et al*. (1989)
Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	–	10000	Brusick *et al*. (1989)
Chromosomal aberrations, rat-kangaroo kidney cells *in vitro*	–	NT	200	Green *et al*. (1970)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	250	Stone *et al*. (1969b)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	4000	Jemison *et al* (1984)
Micronucleus formation, mouse bone marrow *in vivo*	–		2500 × 5 ip	Bruce & Heddle (1979)
Chromosomal aberrations, gerbil bone marrow *in vivo*	+		30 × 5 ip	Majumdar & Solomon (1971)
Chromosomal aberrations, Holzman rat bone marrow *in vivo*	–		500 diet 10 mo	Friedman *et al*. (1972)
Chromosomal aberrations, Holzman rat spermatogonia *in vivo*	–		500 diet 10 mo	Friedman *et al*. (1972)
Dominant lethal mutation, ICR/Ha Swiss mice *in vivo*	–		1000 × 5 po	Epstein *et al*. (1972)
Dominant lethal mutation, (C3H × 101) F_1 mice *in vivo*	–		4000 × 30 po	Cain *et al*. (1988)
Dominant lethal mutation, Osborne-Mendel rats *in vivo*	–		1000 diet 10 mo	Friedman *et al*. (1972)

Table 1 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Heritable translocation, mice *in vivo*	–		4000 × 30 po	Cain *et al.* (1988)
Sperm morphology, (C57BL/6 × C3H/He) F_1 mice *in vivo*	–		500 × 5 ip	Wyrobek & Bruce (1975)
Sperm morphology, (C57BL/6 × C3H/He) F_1 mice *in vivo*	–		2500 × 5 ip	Bruce & Heddle (1979)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneal; mo, month; po, oral

Table 2. Genetic and related effects of sodium cyclamate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Haworthia variegata, chromosomal aberrations	–	NT	20000	Majumdar & Lane (1970)
Drosophila melanogaster, sex-linked mutation	–		2 µg/fly inj	Šrám & Ondrej (1968) [abst]
Drosophila melanogaster, sex-linked mutation	–		5000	Vogel & Chandler (1974)
Drosophila melanogaster, aneuploidy	–		160 mg/mL feed	Félix & de la Rosa (1971)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	NT	250	Kristoffersson (1972)
Cell transformation, Fischer 344 rat bladder explant *in vitro*	+	NT	240	Nicholson & Jani (1988)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	7500	Wolff (1983)
Chromosomal aberrations, human fibroblasts *in vitro*	+	NT	200	Stone *et al*. (1969b)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	250	Stone *et al*. (1969b)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	200	Stoltz *et al*. (1970)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	5000	Collin (1971)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	2000	Tokomitsu (1971)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	4500	Perez Requejo (1972)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	20	Shamberger *et al*. (1973)
Host-mediated assay, *Salmonella typhimurium* in NMRI mice	–		500	Buselmaier *et al*. (1972)
Chromosomal aberrations, BALB/c mice spermatogonia *in vivo*, spermatocytes observed	–		3400 drink 150 d	Leonard & Linden (1972)
Chromosomal aberrations, Chinese hamster spermatogonia *in vivo*	–		2000 × 5 po	Machemer & Lorke (1975a)
Dominant lethal mutation, NMRI mice *in vivo*	–		10000 × 5 po	Lorke (1973)
Dominant lethal mutation, mice *in vivo*	–		2000 diet 70 d	Lorke & Machemer (1975)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal mutation, mice *in vivo*	–		10000 × 1 po	Machemer & Lorke (1975b)
Sperm morphology, (CBA × BALB/c) F_1 mice *in vivo*	–		1000 × 5 ip	Topham (1980)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw per day; d, day; inj, injection; po, oral; ip, intraperitoneal

Table 3. Genetic and related effects of cyclohexylamine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	–	NT	NR	Mayer *et al*. (1969)
Salmonella typhimurium TA100, TA98, TA1535 TA1537, reverse mutation	–	–	2500 µg/plate	Herbold (1981)
Salmonella typhimurium TA100,, TA98, TA1535, TA1537, reverse mutation	–	–	10000 µg/plate	Mortelmans *et al*. (1986)
Salmonella typhimurium TA100, TA98, TA1535, TA1538, reverse mutation	NT	–	2500 µg/plate	Anderson & Styles (1978)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		990 µg/mL feed	Vogel & Chandler (1974)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		1720 µg/mL feed	Brusick *et al*. (1989)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		0.2% feed or 1 mg/fly inj	Knaap *et al*. (1973)
Drosophila melanogaster, heritable translocations	–		NR	Knaap *et al*. (1973)
Drosophila melanogaster, aneuploidy	–		6880 ppm feed	Félix & de la Rosa (1971)
Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	–	NT	860	Brusick *et al*. (1989)
Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	–	1376	Brusick *et al*. (1989)
Chromosomal aberrations, rat-kangaroo kidney cells *in vitro*	+	NT	50	Green *et al*. (1970)
Cell transformation, SA7/Syrian hamster embryo cells	+	NT	62	Casto (1981)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	99	Wolff (1983)
Chromosomal aberrations, human lymphocytes *in vitro*	(+)	NT	99	Stoltz *et al*. (1970)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	500	Brewen *et al*. (1971)
Host-mediated assay, *Salmonella typhimurium* G46 and *Serratia marcescns* a21 in mouse peritoneal cavity	–	NT	100	Buselmaier *et al*. (1972)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Host-mediated assay, human leukocytes in Chinese hamster peritoneal cavity	–	NT	450 ip × 1	Brewen *et al*. (1971)
Mouse spot test	(+)		200 ip × 1	Fahrig (1982)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		50 ip × 5	Legator *et al*. (1969)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	–		50 po/ip × 5	Dick *et al*. (1974)
Chromosomal aberrations, fetal sheep bone-marrow cells *in vivo*	–		250 iv × 1	Turner & Hutchinson (1974)
Chromosomal aberrations, Chinese hamster bone-marrow *in vivo*	–		450 ip × 1	Brewen *et al*. (1971)
Chromosomal aberrations, rat leukocytes *in vivo*	–		50 ip × 35	Mostardi *et al*. (1972)
Chromosomal aberrations, fetal sheep leukocytes *in vivo*	+		50 iv × 1	Turner & Hutchinson (1974)
Chromosomal aberrations, Chinese hamster leukocytes *in vivo*	+		200 po × 3	Van Went-deVries *et al*. (1975)
Chromosomal aberrations, mouse spermatogonia *in vivo*, spermatocytes observed	–		100 ip × 5	Cattanach & Pollard (1971)
Chromosomal aberrations, rat spermatogonia treated *in vivo*, spermatogonia observed	+		50 ip × 1	Legator *et al*. (1969)
Chromosomal aberrations, Chinese hamster spermatogonia treated *in vivo*, spermatogonia observed	–		102 po × 5	Machemer & Lorke (1976)
Dominant lethal mutation, ICR/Ha Swiss mice *in vivo*	–		25 ip × 1	Epstein *et al*. (1972)
Dominant lethal mutation, male mice *in vivo*	–		100 ip × 5	Cattanach & Pollard (1971)
Dominant lethal mutation, mice *in vivo*	+		100 ip × 5	Petersen *et al*. (1972)
Dominant lethal mutation, male NMRI mice *in vivo*	–		102 po × 5	Lorke & Machemer (1975)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
Dominant lethal mutation, male and female mice *in vivo*	–		0.11% diet equival. to 136 mg/kg/d, 10 wk	Lorke & Machemer (1975)
Dominant lethal mutation, male mice *in vivo*	–		150 po × 1	Machemer & Lorke (1975b)
Dominant lethal mutation, male and female rats *in vivo*	– (dec. fert.)		145 po × 3 wk[c]	Khera & Stoltz (1970)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; dec. fert., decreased fertility

[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, *in-vitro* tests, µg/mL; *in-vivo* tests, mg/kg bw per day: NR, not reported; inj, injection; ip, intraperitoneal; po, oral; iv, intravenous; d, day; wk, week

[c] Male and female rats treated with 2% cyclohexylamine in drinking-water for three weeks. Males were then gavaged daily with 220 mg/kg for four weeks followed by three weeks without treatment then two more weeks of gavage treatment; each male was mated with two females on five days/week throughout the treatment period.

Results from a single study showed a high incidence of epithelial foci in Fischer 344 rat bladder explants exposed to sodium cyclamate. Chromosomal aberrations were induced in Chinese hamster embryonic lung cells cultured with sodium cyclamate in one study, in human skin fibroblasts cultured with sodium cyclamate in another study, and in human lymphocytes treated with sodium cyclamate *in vitro* in five studies. Sister chromatid exchange was induced in human lymphocyte cultures exposed to sodium cyclamate in a single study.

Gene mutation was not induced in a host-mediated assay in which mice were injected intraperitoneally with *S. typhimurium* strain G46 and exposed to sodium cyclamate by subcutaneous injection. Chromosomal aberrations were not induced in the spermatogonia of mice or Chinese hamsters exposed to sodium cyclamate in drinking-water for 150 days or by gavage for five consecutive days, respectively. Dominant lethal mutations were not induced in male and female mice fed a diet containing sodium cyclamate for 10 weeks or in male mice treated orally for five days or female mice dosed once. Sperm morphology was unaffected in mice treated by intraperitoneal injection on five consecutive days with sodium cyclamate.

Cyclohexylamine

Cyclohexylamine did not induce bacterial prophage in *E. coli* or gene mutations in *S. typhimurium*. It did not induce somatic cell mutation or recombination, sex-linked recessive lethal mutation, heritable translocation or aneuploidy in *D. melanogaster*. *In vitro*, it did not induce unscheduled DNA synthesis in rat hepatocytes or gene mutations in Chinese hamster ovary cells, but it did induce chromosomal aberrations in rat-kangaroo kidney cells and transformation of Syrian hamster embryo cells. In human lymphocyte cultures, cyclohexylamine induced sister chromatid exchange, but the frequency of chromosomal aberrations was increased only slightly in one study and not at all in another. After intraperitoneal injection of cyclohexylamine, gene mutations were not induced in host-mediated assays in bacteria in the peritoneal cavity of mice or in human lymphocytes in the peritoneal cavity of Chinese hamsters. *In vivo*, cyclohexylamine was weakly mutagenic in one mouse spot test. While the frequency of chromosomal aberrations was increased in one of two studies in rat bone marrow, no aberrations were seen in the bone marrow of Chinese hamsters or fetal sheep, nor in rat leukocytes. Chromosomal aberrations were induced by cyclohexamine in leukocytes of Chinese hamsters and fetal sheep. The compound also induced chromosomal aberrations in rat spermatogonia when these cells were recovered shortly after exposure *in vivo*, but there was no increase in the frequency of chromosomal aberrations in mouse spermatocytes when their precursors had been exposed to cyclohexylamine during the spermatogonial stage. Dominant lethal effects were enhanced in mice in one study, while there was no effect in five other studies. In a single study in rats, cyclohexylamine did not induce dominant lethal mutations.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Cyclamates are widely used as non-caloric sweeteners, the average daily dietary intake generally being less than 3 mg/kg bw.

5.2 Human carcinogenicity data

Use of cyclamates was analysed separately in only four of the studies summarized in the monograph on saccharin and its salts. No increase in the risk for urinary bladder cancer was seen.

5.3 Animal carcinogenicity data

Sodium cyclamate was tested by oral administration in two experiments in mice, one of which was a multigeneration study, and in three experiments in rats. No treatment-related increase in tumour incidence was found. Sodium cyclamate was also tested by oral administration in other experiments in mice, rats, hamsters and monkeys, but these experiments could not be evaluated because of various inadequacies or incomplete reporting.

Pellets containing sodium cyclamate induced bladder tumours in mice after implantation into the bladder; however, the protocol was considered to be inadequate for determining carcinogenicity.

Calcium cyclamate was tested by oral administration in a two-generation experiment in rats; no difference in tumour incidence was seen between treated and control animals.

In two studies in rats, sodium cyclamate was administered orally after a known carcinogen. The incidence of urinary bladder tumours was increased in one study, whereas only slight enhancement was found in a second study.

5.4 Other relevant data

Cyclamates are incompletely absorbed from the gastrointestinal tract of humans and other mammals. Most is excreted in the urine unchanged. Cyclamates are partially converted by gastrointestinal microflora to cyclohexylamine, which is absorbed. Cyclamates and cyclohexylamine can produce testicular toxicity in rats.

Cyclamates have not been observed to produce developmental toxicity in mice, rats, rabbits, hamsters, dogs or rhesus monkeys. Negative results were obtained in a number of short-term assays for teratogenicity *in vitro* and *in vivo*. Cyclamates were not teratogenic in mice, rats or rhesus monkeys, but there were some indications of reduced growth and viability of embryos in some studies. Rat embryos exposed *in vitro* showed altered morphological development in one study.

Cyclamates did not produce chromosomal aberrations in peripheral lymphocytes of volunteers. Cyclamates were not genotoxic in rodents *in vivo* but were genotoxic in mammalian cells *in vitro*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of cyclamates.

There is *inadequate evidence* in experimental animals for the carcinogenicity of cyclamates.

Overall evaluation

Cyclamates are *not classifiable as to their carcinogenicity to humans (Group 3)*.

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