Long-Term Toxicity and Carcinogenicity Study of Cyclamate in Nonhuman Primates


*Division of Basic Sciences, National Cancer Institute, Bethesda, Maryland 20892; †Clinical Pharmacology Group, University of Southampton, Biomedical Sciences Building, Bassett Crescent East, Southampton SO16 7PX, United Kingdom; ‡Department of Pathology and Microbiology and the Eppley Institute for Research on Cancer and Allied Diseases, University of Nebraska, Omaha, Nebraska; §Nara Medical University, Kashihara, Nara 634, Japan; ¶Covance Laboratories America, Inc., Vienna, Virginia 22182; ||Office of the Director, National Cancer Institute, Bethesda, Maryland 20892.

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Twenty-one monkeys (cynomolgus, rhesus, African green) were fed cyclamate (100 mg/kg and 500 mg/kg) in the diet five times per week from a few days after birth and continuing for up to 24 years. Malignant tumors were diagnosed in three 24-year-old cyclamate monkeys; these were metastatic colon carcinoma (rhesus; 500 mg/kg), metastatic hepatocellular carcinoma (cynomolgus; 500 mg/kg), and a small, well differentiated adenocarcinoma of the prostate (cynomolgus; 100 mg/kg). Benign tumors were found at necropsy in three females; these were adenoma of the thyroid gland (rhesus; 100 mg/kg) and two cases of leiomyoma of the uterus (rhesus; 100 mg/kg and 500 mg/kg). No tumors were detected in an age-matched control group of 16 monkeys. Examination of the testes revealed complete testicular atrophy in one of the old cyclamate monkeys, and focal germ cell aplasia (Sertoli-only tubules) in two other cyclamate monkeys. Focal spermatogenic interruption (maturation arrest) at various germ cell levels mixed with normal spermatogenesis was observed in both the cyclamate-treated and the control monkeys, all of which were over 20 years old. Measurements of terminal cyclohexylamine concentrations showed that three of the males dosed with cyclamate at 500 mg/kg were high converters, with plasma concentrations comparable to the levels that produce testicular atrophy in rats. However, only one of the three high converters showed histologic evidence of irregular spermatogenesis. The overall conclusion is that the testicular abnormalities and the sporadic cases of different malignancies found after more than 20 years of dosing do not provide clear evidence of a toxic or carcinogenic effect of sodium cyclamate in monkeys.

Key Words: cyclamate; primates; carcinogen; toxicity; testicular abnormalities; malignancies; spermatogenesis.

Sodium cyclamate was first marketed in 1951 when it was approved by the U.S. Food and Drug Administration (Bopp et al., 1986). In 1970, a report by Price et al. on an increased incidence of bladder tumors in rats dosed with a mixture of sodium cyclamate and sodium saccharin (Price et al., 1970) led to a ban on the use of cyclamate as an artificial sweetener in a number of countries, including the United Kingdom and the United States. Based on subsequent toxicity and carcinogenicity studies, a report was published by a review panel at the National Cancer Institute concluding that it could not be established that cyclamate was not carcinogenic in experimental animals (DHEW publication, 1976). Following criticism of this review (see Bopp et al., 1986), the carcinogenicity data were evaluated by Cancer Assessment Committee of the Center for Food Safety and Applied Nutrition of the FDA, and also by a joint National Academy of Sciences – National Research Council Committee (NAS-NRC, 1985); the carcinogenicity of cyclamate has also been evaluated by the FAO-WHO Joint Expert Committee on Food Additives (JECFA) and by the Scientific Committee for Foods (SCF) of the European Union. These recent evaluations have concluded that cyclamate is not a carcinogen. Cyclamate has continued to be approved in many countries worldwide, and the acceptable daily intake (ADI) of 0–11 mg/kg/day by JECFA and SCF has been maintained for the past decade. The SCF is currently evaluating new data on the safety of cyclamate.

Cyclamate is converted to the more toxic metabolite cyclohexylamine by the bacterial flora of the gastrointestinal tract (Renwick, 1986). In rats, testis is the organ most sensitive to the toxic effects of cyclohexylamine. Testicular atrophy, characterized by reduction in testicular weight and marked impairment of spermatogenesis has been reported in rats (Gaunt et al., 1974 and 1976; Oser et al., 1976; Roberts et al., 1989) and dogs (James et al., 1981) but not mice (Roberts et al., 1989). A comprehensive pair-feeding study has shown a no-observed-adverse-effect level (NOAEL) for testicular atrophy in rats at 100 mg/kg/day, with a clear dose-response relationship at 200 mg/kg/day and above (see Bopp et al., 1986, for details). The clearance of cyclohexylamine is more rapid in mice than in rats (Roberts and Renwick, 1989), which may explain why testicular toxicity has not been reported in mice (Bopp et al., 1986). Testicular atrophy has also been reported following chronic administration of sodium cyclamate in the diet (mostly at
5–10%) to rats (Ferrando and Huchet, 1968; Nees and Derse, 1965; Oser et al., 1975). The atrophy was seen at the end of the chronic studies and associated with marked reductions in body weight and the weights of other organs. The effects in elderly rats could be due to metabolism of cyclamate to cyclohexylamine, but this would have required sustained and high levels of metabolism (>10%; see Bopp et al., 1986), which is unlikely in rat colonies (Bickel et al., 1974; Oser et al., 1975; Renwick, 1986).

Cyclamone conversion to cyclohexylamine has also been studied extensively in humans (Bopp et al., 1986; Buss et al., 1992; Collings, 1989; Renwick, 1986). There are major inter- and intraspecies variations in the extent to which the proportion of an oral dose of cyclamate that is not absorbed from the gastrointestinal tract is converted to cyclohexylamine by the intestinal flora (Renwick, 1986). The ADI for cyclamate established by the JECFA and SCF (1985) is based on the NOAEL for testicular atrophy produced by cyclohexylamine in rats with the application of a 100-fold safety factor and assuming 18% metabolism per day (see Renwick, 1986).

This report presents results of a toxicity and carcinogenicity study of cyclamate in nonhuman primates that involved treatment for a period of up to 24 years, i.e., from 1970 to 1994.

**MATERIALS AND METHODS**

**Animals and dosing.** Of the 21 monkeys used in this study, there were nine (seven males, two females) cynomolgus *(Macaca fascicularis)*, nine (four males, five female) rhesus *(Macaca mulatta)*, and three (two males, one female) African green *(Cercopithecus aethiops)* monkeys. These three species had been used in earlier chemical carcinogenesis studies in this monkey colony and had exhibited comparable sensitivity to different test compounds with respect to tumor incidence, latent period, and tumor type (Thorsteinsson et al., 1994). They were born and raised in a closed colony. Infants were hand-reared and began receiving cyclamate (a gift from Abbott Laboratories, Chicago, IL) in the Similac formula within the first few days of birth. At 6 months of age, the monkeys were placed in individual, wall-mounted, stainless steel cages equipped with an automatic watering device. The daily diet consisted of *ad libitum* Purina High Protein Monkey Chow #5045, a vitamin sandwich, and half an apple. The sodium cyclamate (dissolved in warm water at 200 mg/ml) was incorporated into the vitamin mixture, which consisted of the following ingredients: powdered milk (5 pounds); Parvo (folic acid supplement, 4 oz., 20% with starch, Roche Agricultural Products); Cecon (vitamin C supplement, 300 ml, Abbott Laboratories, Chicago, IL); molasses (2 l); and water (500 ml). The appropriate amounts of the vitamin mixture containing sodium cyclamate (0.5 ml/animal for the 100 mg/kg group and 2.5 ml/animal for the 500 mg/kg group) were placed onto sandwiches. The monkeys (four cynomolgus, four rhesus, two African green) in the 100 mg/kg group received one sandwich daily every 6 days, whereupon each monkey was dosed by veterinary every 6 months when blood was drawn for routine hematologic tests, electrolyte measurements, and liver function tests. Tuberculin tests were performed on a quarterly basis.

In 1994, a decision was made to end the cyclamate study and euthanize the remaining 14 cyclamate and 16 control monkeys. Complete necropsies were carried out on all of the animals. The following organs were removed and fixed in buffered formalin solution: brain, pituitary, salivary glands, thyroid, thymus, tongue, cheek pouches, trachea, esophagus, lungs, heart, aorta, liver, gallbladder, pancreas, spleen, kidneys, adrenals, stomach, duodenum, small intestine, colon, urinary bladder, uterus, ovaries, testes, seminal vesicles, prostate, mammary tissue, lymph nodes (axillary, inguinal, hilar), skin, skeletal muscle, and bone (sternum). Samples of tissues were embedded in paraffin and histologic sections were stained with hematoxylin-eosin (H&E). Giemsa and PAS staining was also performed on the sections of the testes.

**Plasma, urine, and tissue samples analysis.** Samples of plasma, urine, and tissues were collected, frozen, and transported to the University of Southampton, U.K., where they were analyzed for cyclohexylamine using the method of Buss et al., 1992. The samples were analyzed in duplicate; samples showing poor replicate values (>5% difference) were subject to further analyses to confirm the results. Triple replicate standard curves of 3–5 different concentrations of cyclohexylamine spiked into the biologic matrix from control animals were analyzed with the samples; these were linear over the ranges studied (0–2 μg/ml for plasma; 0–10 μg/ml for urine; 0–10 μg/ml homogenate for testes) and showed intra-assay variability of 7% or less at each concentration. The sample data represent the means of 2–4 replicate values mostly within ±5%.

The urine samples showed a very wide range of concentrations of cyclohexylamine, so that they had to be diluted to different extents—up to 1 in 100. In each case, 0.5 ml of the final dilution was used for analysis. The testicular samples were homogenized in 0.1 M phosphate buffer, and aliquots were extracted and analyzed. Samples from control monkeys showed a small interfering peak on HPLC that corresponded to low concentrations of cyclohexylamine (see Results).

**RESULTS**

This study included 21 cyclamate monkeys and 16 age-matched controls (see Tables 1–3). The monkeys were dosed from a few days after birth until the time of death. In 1994 when a decision was made to terminate the study, 7 cyclamate-treated monkeys had died and 14 were still alive. A cyclamate-treated monkey (789J; 500 mg/kg dose) died accidentally at 2 years of age and did not show any specific abnormalities when necropsied. A second monkey (790J; 500 mg/kg dose) died at 7 years of age and showed renal tubular degeneration. No other problems were documented until 1985, when two monkeys (769J; 100 mg/kg, and 800J; 500 mg/kg) died during a vari-cellula outbreak. The same year, one monkey died as a result of chronic myocarditis (782J; 500 mg/kg) and two females were euthanized due to severe symptoms from extensive pelvic endometriosis (773J; 100 mg/kg, and 795J; 500 mg/kg). When the cyclamate study was terminated in 1994, the remaining eight monkeys in the 100 mg/kg group and six in the 500 mg/kg group, as well as the controls, were euthanized and necropsied. At that time, kyphosis was observed in three of the cyclamate monkeys. No other external abnormalities or health problems were noted. Results of blood samples collected from these animals did not reveal any abnormalities in blood cell counts, liver function tests, electrolytes, or BUN (results not shown).
Tumor Incidence

In 1994, two of the 24-year-old monkeys in the 500 mg/kg dose group were diagnosed with advanced cancer; a female (801J) with metastatic adenocarcinoma of the colon and a male (787J) with metastatic hepatocellular carcinoma (Table 2). The third cancer case was a minute (3^3 mm) well-differentiated papillary adenocarcinoma of the prostate that was found during necropsy of an animal (774J) dosed at 100 mg/kg (Table 1). Benign neoplasms were found during necropsy of two females in the 100 mg/kg cyclamate group and one in the 500 mg/kg group; one was an adenoma of the thyroid (791J) (Table 1) and two were leiomyomas of the uterus (772J and 7801J) (Tables 1 and 2). No neoplasms were detected in the control animals.

Testicular Findings

Evaluation of testicular function was carried out in 1982, after 12 years of dosing, in twelve cyclamate-treated monkeys (numbers 786J, 770J, 771J, 774J, 782J, 784J, 785J, 786J, 787J, 799J, 800J, 943M) and the age-matched controls. Semen analysis and measurements of serum testosterone and gonadotropin levels did not reveal any differences between the cyclamate groups and the age-matched controls (data not shown). In addition, testicular biopsies from these monkeys showed normal histology. In 1990, the right testis was removed from one of the monkeys (943M) in the 100 mg/kg cyclamate group during a repair of an inguinal hernia. Microscopic examination of the testis showed areas of atrophic tubules and other areas showing normal spermatogenesis, and in view of the fact that this animal was in relatively poor health for many years due to chronic gastrointestinal problems, the testicular atrophy may not have been related to cyclamate exposure. Two monkeys (784J and 786J ) in the 500 mg/kg cyclamate group displayed focal germ cell aplasia (Sertoli-cell only tubules) mixed with areas showing normal spermatogenesis. Monkey number 784J also showed evidence of chronic orchitis. The consensus by the pathologists was that the testicular changes in the three cyclamate-treated monkeys listed above are probably not related to cyclamate exposure, as the remainder of the cyclamate group did not differ from the controls.

Other Histologic Findings

The pathologic findings of the two cyclamate groups and the control animals are shown in Tables 1, 2, and 3. Because sodium cyclamate has been implicated as a bladder carcinogen in rodents, special attention was given to macro- and microscopic examination of the urinary bladder. No premalignant lesions of the urothelium or bladder tumors were detected in either the cyclamate or control groups. Similarly, light and sections were examined independently by three pathologists. Focal spermatogenic arrest was seen in representative sections from several animals in both the cyclamate and control groups. In these cases, many areas showed normal spermatogenesis, and mature sperms were present in the epididymis. One monkey (771J) in the 100 mg/kg cyclamate group showed severe atrophy of the right testis, but a biopsy taken from the same testis in 1984 had shown normal spermatogenesis; the left testis had been removed from this monkey in 1990 during a repair of a long-existing inguinal hernia. Microscopic examination of the left testis showed areas of atrophic tubules and other areas showing normal spermatogenesis, and in view of the fact that this animal was in relatively poor health for many years due to chronic gastrointestinal problems, the testicular atrophy may not have been related to cyclamate exposure. Two monkeys (784J and 786J) in the 500 mg/kg cyclamate group displayed focal germ cell aplasia (Sertoli-cell only tubules) mixed with areas showing normal spermatogenesis. Monkey number 784J also showed evidence of chronic orchitis. The consensus by the pathologists was that the testicular changes in the three cyclamate-treated monkeys listed above are probably not related to cyclamate exposure, as the remainder of the cyclamate group did not differ from the controls.
electron microscopic examination of the kidneys did not reveal any differences between the groups (data not shown). As shown in Tables 1, 2, and 3, hyalinization of the pancreatic Langerhans islets was observed in two cyclamate-treated animals and three controls. This was associated with high blood glucose levels in one of the cyclamate-treated monkeys in the 500 mg/kg group (784J) and one of the control monkeys (678H). Hyalinization of the Langerhans islets has been observed sporadically in breeders and animals dosed with different test compounds in this monkey colony. The pathogenesis of this finding is unknown.

Cyclohexylamine Levels in Plasma, Urine, and Testes

Terminal cyclohexylamine levels were determined in plasma, urine, and testes, as described in the Materials and Methods section. Because of the presence of slight interference, the apparent cyclohexylamine concentrations in control

### TABLE 2
Pathologic Findings in Monkeys Administered 500 mg/kg/day Cyclamate

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Species</th>
<th>Sex</th>
<th>Terminal body wt. (kg)</th>
<th>Age at first dose</th>
<th>Months dosed</th>
<th>Cumulative dose (g)</th>
<th>Pathologic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>782J</td>
<td>R</td>
<td>M</td>
<td>12.2</td>
<td>N/A</td>
<td>183</td>
<td>17010</td>
<td>Chronic myocarditis</td>
</tr>
<tr>
<td>784J</td>
<td>C</td>
<td>M</td>
<td>7.1</td>
<td>1 day</td>
<td>288</td>
<td>17158</td>
<td>Focal germ cell aplasia (Sertoli-cell only tubules) of testes; hyalinization of pancreatic Langerhans islets; high blood glucose level; kyphosis</td>
</tr>
<tr>
<td>785J</td>
<td>C</td>
<td>M</td>
<td>6.2</td>
<td>1 day</td>
<td>287</td>
<td>16910</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>786J</td>
<td>R</td>
<td>M</td>
<td>9.5</td>
<td>1 day</td>
<td>287</td>
<td>19123</td>
<td>Chronic orchitis; focal germ cell aplasia (Sertoli-cell only tubules)</td>
</tr>
<tr>
<td>787J</td>
<td>C</td>
<td>M</td>
<td>5.9</td>
<td>2 days</td>
<td>281</td>
<td>19415</td>
<td>Metastatic hepatocellular carcinoma</td>
</tr>
<tr>
<td>789J</td>
<td>G</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>24</td>
<td>92</td>
<td>Aspiration of vomitus</td>
</tr>
<tr>
<td>790J</td>
<td>R</td>
<td>F</td>
<td>3.2</td>
<td>4 days</td>
<td>84</td>
<td>1851</td>
<td>Renal tubular degeneration</td>
</tr>
<tr>
<td>795J</td>
<td>R</td>
<td>F</td>
<td>5.0</td>
<td>1 day</td>
<td>189</td>
<td>12015</td>
<td>Extensive endometriosis</td>
</tr>
<tr>
<td>799J</td>
<td>C</td>
<td>M</td>
<td>6</td>
<td>1 day</td>
<td>285</td>
<td>17178</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>800I</td>
<td>C</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>172</td>
<td>10048</td>
<td>Systemic varicella infection</td>
</tr>
<tr>
<td>801J</td>
<td>R</td>
<td>F</td>
<td>6.5</td>
<td>1 day</td>
<td>281</td>
<td>16917</td>
<td>Metastatic adenocarcinoma of colon; leiomyoma of uterus</td>
</tr>
</tbody>
</table>

Note. F, female; M, male; N/A, not available. C, cynomolgus; R, rhesus; G, green.

### TABLE 3
Summary of Data from Control Monkeys

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Species</th>
<th>Sex</th>
<th>Terminal body wt. (kg)</th>
<th>Observation (mos)</th>
<th>Pathologic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>678H</td>
<td>C</td>
<td>F</td>
<td>4.20</td>
<td>301</td>
<td>High blood glucose level; hyalinization of pancreatic Langerhans islets; hydronephrosis; endometriosis</td>
</tr>
<tr>
<td>697I</td>
<td>R</td>
<td>M</td>
<td>9.60</td>
<td>261</td>
<td>Pulmonary emphysema; diverticulitis of the colon</td>
</tr>
<tr>
<td>777J</td>
<td>R</td>
<td>M</td>
<td>7.50</td>
<td>289</td>
<td>Bloat; cyst, left kidney</td>
</tr>
<tr>
<td>778J</td>
<td>R</td>
<td>M</td>
<td>5.50</td>
<td>284</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>779J</td>
<td>R</td>
<td>F</td>
<td>5.50</td>
<td>299</td>
<td>Chronic gastritis; chronic cholecystitis</td>
</tr>
<tr>
<td>780J</td>
<td>C</td>
<td>M</td>
<td>4.90</td>
<td>285</td>
<td>Hyalinization of pancreatic Langerhans islets</td>
</tr>
<tr>
<td>899L</td>
<td>C</td>
<td>M</td>
<td>6.30</td>
<td>268</td>
<td>Chronic bronchitis</td>
</tr>
<tr>
<td>944M</td>
<td>R</td>
<td>M</td>
<td>9.80</td>
<td>237</td>
<td>Pulmonary edema; inguinal hernia</td>
</tr>
<tr>
<td>947M</td>
<td>R</td>
<td>M</td>
<td>12.20</td>
<td>234</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>987M</td>
<td>C</td>
<td>F</td>
<td>4.90</td>
<td>240</td>
<td>Congenital absence of right kidney with compensatory hypertrophy of left kidney; endometriosis</td>
</tr>
<tr>
<td>989M</td>
<td>R</td>
<td>M</td>
<td>8.50</td>
<td>257</td>
<td>Nodular cortical hyperplasia, left adrenal gland</td>
</tr>
<tr>
<td>1017N</td>
<td>C</td>
<td>M</td>
<td>4.90</td>
<td>244</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>1041N</td>
<td>C</td>
<td>M</td>
<td>7.50</td>
<td>248</td>
<td>Hyalinization, pancreatic Langerhans islets</td>
</tr>
<tr>
<td>1156R</td>
<td>R</td>
<td>F</td>
<td>5.00</td>
<td>224</td>
<td>No specific abnormalities</td>
</tr>
<tr>
<td>1188S</td>
<td>C</td>
<td>M</td>
<td>5.90</td>
<td>217</td>
<td>Hyalinization, pancreatic Langerhans islets</td>
</tr>
<tr>
<td>1234T</td>
<td>C</td>
<td>F</td>
<td>4.60</td>
<td>206</td>
<td>No specific abnormalities</td>
</tr>
</tbody>
</table>

Note. F, female; M, male. C, cynomolgus; R, rhesus.
monkey plasma ranged from 0.07 to 0.23 μg/ml respectively (Table 4). Given the terminal plasma concentrations in the controls, cyclamate monkeys 765J, 768J, 770J, and 795J were rated as nonconverters (−) (0.23 μg/ml); 771J, 782J, 790J, 791J, and 943M as low converters (+) (0.23–1.0 μg/ml); 772J and 773J as good converters (+++) (1.0–5.0 μg/ml); and 786J, 787J, and 799J as high converters (++++) (> 5 μg/ml). The three high converters (which belonged to the 500 mg/kg group) had the highest testicular and urine cyclohexylamine levels (Table 4). Interestingly, monkey 786J was one of two cyclamate-treated animals that showed focal germ cell aplasia (Sertoli-cell only tubules) of the testes.

**DISCUSSION**

The present study evaluated the long-term toxic and carcinogenic effects of sodium cyclamate on three monkey species. Of the 21 monkeys that were started on cyclamate as newborns in 1970, 14 survived until the study was terminated in 1994. In view of reports on cyclohexylamine- and cyclamate-induced lesions in rats, special attention was given to the examination of the testes and urinary bladders. There were no adverse effects on the urinary bladders or clear spermatogenic irregularities associated with the long-term cyclamate exposure in these old animals. One case of early prostate cancer and two cases of benign tumors (thyroid adenoma and uterine leiomyoma) were found in the 100 mg/kg cyclamate group. Two cases of metastatic cancer (one colon carcinoma and one hepatocellular carcinoma) and one case of a leiomyoma were diagnosed in 24-year-old monkeys in the 500 mg/kg cyclamate group.

The increased incidence of bladder tumors was reported in rats fed with high dietary concentrations of a mixture of sodium cyclamate and sodium saccharin (Price *et al.*, 1970) and led to the banning of cyclamate in the United States (U.S. Food & Drug Administration, 1970). Although cyclamate was associated with bladder tumors in another chronic feeding study (Oser *et al.*, 1975), the effect could not be reproduced in a number of other cyclamate carcinogenicity bioassays (Bopp, 1986). In contrast, chronic dosing with very high dietary concentrations of sodium saccharin and a variety of sodium salts consistently promote carcinogenesis in the rat bladder (Cohen...
et al., 1995). However, a long-term feeding study of sodium saccharin showed no evidence of carcinogenic effect on the urinary tract in nonhuman primates (Takayama et al., 1998). The lack of urothelial abnormalities in the saccharin and cyclamate-treated monkeys supports epidemiologic data that suggest that sodium cyclamate and sodium saccharin are not human bladder carcinogens (IARC, 1980; WHO, 1993).

Three cases of different types of malignant tumors were found in the cyclamate monkeys. One case was a minute, well-differentiated prostate carcinoma that was detected at the time of necropsy of a cynomolgus monkey in the 100 mg/kg group. The other two cases (a colon carcinoma and a hepatocellular carcinoma) occurred in the 500 mg/kg group. Both monkeys presented with widely disseminated malignancies at 24 years of age. No malignant tumors were detected in the monkeys presented with widely disseminated malignancies at 24 years of age. No malignant tumors were detected in the age-matched control group, however. We have previously reviewed the incidence of spontaneous tumor development in the breeders and control animals in this monkey colony since its onset in 1961 (Thorgeirsson et al., 1994). The spontaneous malignant tumor rate among 373 monkeys studied was 1.5% in cynomolgus, 2.8% in rhesus, and 8% in African green monkeys. Because statistical analysis could not be adequately carried out on the limited number of animals per group in this study, it is possible that a weak carcinogenic effect of cyclamate could be missed. However, the following findings would argue against cyclamate being carcinogenic: monkeys developed different types of malignant tumors, i.e., carcinomas of liver, colon, and prostate; tumors were detected after more than 20 years of dosing; tumors developed in both the 100 mg/kg and 500 mg/kg cyclamate groups; no proliferative or premalignant lesions were detected in the liver, colon, or prostate of the remaining cyclamate monkeys.

Cyclamate conversion to the toxic metabolite cyclohexylamine has been studied extensively in different experimental animal species as well as in humans (Bopp et al., 1986; Buss et al., 1992; Collings, 1989; Renwick, 1986). The testis is the organ most sensitive to the toxicologic effects of cyclohexylamine in rats. A comparative study was carried out on the effects of cyclohexylamine in rats and cynomolgus monkeys and reviewed by the SCF (1995). A daily intake of 100 mg/kg of cyclohexylamine for 4 weeks produced testicular toxicity in the monkeys. However, 34 mg/kg (equivalent to approximately 200 mg/kg in rats, based on plasma concentration) for the same dosing period produced only slight changes in water intake and body weight but no histologic changes of the testes. The formation of cyclohexylamine was determined in our cyclamate-treated monkeys in 1984 (based on urine collections) and again at euthanasia in 1994. Three monkeys were categorized as high converters in 1994, with plasma cyclohexylamine levels of > 5 µg/ml, and these plasma levels are similar to those in rats showing adverse testicular histology (Roberts and Renwick, 1989). Focal germ cell aplasia (Sertoli-cell only tubules) adjacent to histologically normal seminiferous tubules was observed in two of the 500 mg/kg monkeys (784J; 786J) but not in the control group. Monkey number 786J was a high converter in 1994; 784J was not assessed in 1994, but was a nonconverter in 1984. Sertoli-cell only tubules have also been reported in rats treated with cyclohexylamine (Bopp et al., 1986; Creasy et al., 1990). In considering the focality of these lesions and that they were observed in only two of the cyclamate monkeys, they are considered not to be induced by cyclamate per se, but a role for cyclohexylamine cannot be excluded. The three monkeys that were rated as high converters in 1994 were low or nonconverters when their cyclamate metabolism was determined in 1984; because of the limited number of observations and the fluctuation in cyclamate metabolism, it is impossible to estimate the level of long-term exposure of the monkeys to cyclohexylamine.

During the 24 years of dosing with cyclamate, there was no evidence of abnormal body weight changes. The only external malformation observed in the aged cyclamate monkeys was kyphosis of the thoracic vertebral column in three of the animals. Cyclohexylamine administration has been shown to increase blood pressure in humans (Eichelbaum et al., 1974), but cyclamate did not increase blood pressure, even in subjects with very high conversion to cyclohexylamine at the time of assessment (Buss et al., 1992). This difference may be related to the time-dependent increase in the plasma concentrations of cyclohexylamine (Buss and Renwick, 1992) following dosage with cyclohexylamine per se and cyclamate. The cyclamate monkeys in the present study did not show signs of heart problems and the necropsies did not reveal any specific cardiovascular abnormalities that were different from the controls.

In conclusion, the findings of this study showed that long-term feeding of nonhuman primates with high doses of cyclamate did not affect the general health of most of these animals. Clear evidence of compound-related testicular changes and tumors was not detected in the cyclamate monkeys after more than 20 years of dosing.

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