HUMAN ECOLOGY

Mutagenic Activity of Cyclohexene and Products of Its Chlorination

L. P. Sycheva, Z. I. Zholdakova, E. E. Polyakova, N. E. Lukmanova, L. V. Akhal'tseva, and V. S. Zhurkov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 6, pp. 683-685, June, 2000
Original article submitted January 5, 2000

Cyclohexene exhibits no mutagenic effect in the Ames test. Products of its transformation (chlorination) produced a mutagenic effect in the Ames test on TA 100 strain without metabolic activation and in the micronucleus test on epithelial cells from mouse urinary bladder and colon. These findings are consistent with epidemiological data on higher incidence of urinary bladder and rectal cancers in subjects consuming chlorinated drinking water from surface water reservoirs.

Key Words: *products of cyclohexene chlorination; Ames test; micronucleus test; urinary bladder; colon*

Evaluation of the mutagenic and carcinogenic potential of chlorinated derivatives of some chemical compounds is an important scientific and practical problem. Chlorination is often used in technologies of drinking water production and disinfection of home and industrial waste water. However, epidemiological studies revealed increased prevalence of urinary bladder and colorectal cancer in subjects consuming chlorinated water from surface water reservoirs [5,6,8].

In this work, we evaluated mutagenic effects of cyclohexene chlorination products on mouse bone marrow, colon, and urinary bladder using the Ames and micronucleus tests.

MATERIALS AND METHODS

Cyclohexene was synthesized at the Department of Organic Chemistry of Moscow State University. Cyclohexene was chlorinated in the dark at 25°C in distilled water containing 0.02 M phosphate buffer (pH 6.8-7.0) in a total volume of 2 liters. The reaction mixture contained products of cyclohexene chlorination with chlorine water at 20:1 active chlorine/cyclohexene molar ratio (0.56 g/liter active chlorine and 32.8 mg/liter cyclohexene). The reaction was carried out for 24 h. The solution was then acidified with HCl to pH=2. Chlorinated products were extracted with methylene chloride. The extracts were dried with sodium sulfate anhydride, concentrated in a Claisen flask, to a volume of 1 ml, and dried at room temperature. The dry residue was dissolved in 10 ml sunflower oil for the micronucleus test or in 4 ml DMSO for the Ames test.

The mutagenicity of cyclohexene and products of its chlorination was tested in the Ames test [3] with and without exogenous metabolic activation (EMA) on two strains (TA 100 and TA 98). The S-9 fraction of liver homogenate from male Wistar rats treated with sovol, a microsomal enzyme inducer, was used for metabolic activation. The tests were performed in duplicates, in two dishes, and the means were calculated. Positive control tests were performed with sodium azide for TA 100 without EMA and 2,7-diamino-4,9-

Laboratory of Mutagenesis and Laboratory of Complex Ecological and Hygienic Standardization, A. N. Sysin Institute of Human Ecology and Environmental Hygiene, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** sysen@elnet.msk.ru. Sycheva L. P.

0007-4888/00/0006-581$25.00

dihydroxy-5,10-dioxo-4,5,9,10-tetrahydro-4,9-diazopyrene for TA 98 without EMA. Ethidium bromide was used for verification of metabolic activation on TA 98 with EMA; DMSO and distilled water served as negative controls. The results of mutagenicity tests were considered to be significant if the mean amount of reverted colonies per dish 1.8 or more times surpassed the control for TA 100 and 2 or more times surpassed the control for TA 98.

Animal experiments were performed on 24 male (CBA×C57Bl/6)F1 mice weighing 20 g. The mice were purchased from Kryukovo nursery. Cyclohexene chlorination products (undiluted and diluted 1:1 or 1:3) were administered through a gastric tube in a dose of 0.1 ml per 10 g body weight for 4 consecutive days [3]. Sunflower oil was administered to control mice according to the same protocol. Each group consisted of 6 mice. The animals were killed 24 h after the last gavage.

Bone marrow cells were prepared as recommended elsewhere [4]. Suspensions of urinary bladder and colonic epithelial cells were prepared as described previously [1] with our modifications for the micronucleus test. Fragments of the colon and urinary bladder from each animal were fixed in formalin, washed in running water, and kept in 50% KOH for 16 h. The mucosa and muscular layer were separated with a spatula. The cells were washed in distilled water and centrifuged at 1500 rpm for 7 min. The supernatant was discarded, and smears prepared from the pellet were fixed in ethanol-acetic acid mixture (3:1) for 15 min and stained with 2.5% acetoorcein in a thermostat (37°C) for 1 h. The preparations were examined under a microscope with an immersion objective (10×90). The number of micronucleated cells per 2000 cells from each organ (code-labeled smears) in each mouse was determined. In the bone marrow, the content of polychromatophilic erythrocytes (PCE) in the total erythrocyte population was determined by examining 200 cells. Statistical analysis of the data was performed by the nonparametric Wilcoxon rank test using a Statistica Software.

RESULTS

In the Ames test, cyclohexene was dissolved in DMSO and administered in doses of 0.1, 1, 10, 100, and 1000 μg/dish. The substance caused no mutagenic effect in all variants of the experiments.

In the tests for mutagenicity of cyclohexene chlorination products, the agents were dissolved in DMSO and added in doses of 0.3 and 0.1 ml per dish for the undiluted solution and 0.1 ml for 1:5 and 1:25 dilutions, which was approximately equivalent to the amounts of chemicals in $^1/_{13}$, $^1/_{40}$, $^1/_{200}$, and $^1/_{1000}$ of the total volume (250 ml) of the reaction mixture. The mutagenic effect was produced only by the maximum dose (0.3 ml) of undiluted chlorinated products in the test on TA-100 strain without EMA, which is equivalent to the amount of chemicals in 18.75 ml reaction mixture. We observed no mutagenic effect of transformed products on TA 100 with EMA and TA 98 with or without EMA. The spectrum of mutagenic effects of these products was characteristic of transformed organic contaminants found in chlorinated water from surface water reservoirs [2,7].

Cyclohexene chlorination products caused no mutagenic effect on bone marrow PCE (Table 1). Bone marrow PCE accounted for no more than 0.57 of the total erythrocyte population, which suggested that products of cyclohexene chlorination caused no toxic effect on the bone marrow. A considerable decrease in the micronucleated fraction in the colonic epithelium was found only after administration of 1:1 diluted agent (Table 1), whereas the percentage of micronucleated cells in the urinary bladder epithelium considerably differed from the control after administration of undiluted or 1:3 diluted cyclohexene chlorination products (Table 1).

TABLE 1. Cytogenetic Effects of Cyclohexene Chlorination Products on Bone Marrow, Colon, and Urinary Bladder of (CBA×C57Bl/6)F1 Mice ($X \pm m$, n=6)

Index	Control (sunflower oil)	Cyclohexene chlorination products		
		undiluted	1:1	1:3
Micronucleated PCE, ‰	1.50±0.58	2.00±0.47	1.33±0.36	2.00±0.47
PCE fraction in the total erythrocyte population	0.57	0.53	0.51	0.53
Micronucleated epithelial cells, ‰				
colon	1.33±0.25	2.08±1.14	2.00±0.22*	1.92±0.49
urinary bladder	1.40±0.68	3.58±0.87*	1.17±0.25	4.83±1.33*

Note. *p<0.05 compared to the control.

Thus, mutagenic effects of cyclohexene chlorination products detected by the Ames test were also revealed in experiments on mice. Cyclohexene chlorination products in intragastric administration produced weak, but significant cytogenetic effects more pronounced in the urinary bladder, than in the colon. The induction of mutations in the colon and urinary bladder is consistent with epidemiological data on the higher prevalence of tumors in these organs in populations consuming chlorinated drinking water.

It is important to note that complex toxicological studies should take into account the mutagenic effects occurring not only in the bone marrow, but also in other organs. Accumulation of these data will result in more accurate estimates of the mutagenicity and carcinogenicity of the test substances.

REFERENCES

1. L. N. Belov, M. E. Kogan, M. E. Leont'eva, *et al.*, *Tsitologiya*, **17**, No. 11, 1332-1337 (1975).
2. V. S. Zhurkov, *Humans and Ecology: Express Information*, No. 1-3, Moscow (1993) pp. 25-35.
3. *Methodological Guidelines for Determination of Mutagenicity of Chemicals for Standardization of Their MPC in Water* [in Russian], Moscow (1986).
4. *The Micronucleus Test for Determination of Mutagenicity of Chemicals (Methodological Recommendations)*, Moscow (1984).
5. *IARC Monographs on the Evaluation of Carcinogenic Risks to Human*, Vol. 52, Lyon (1991), pp. 37-141.
6. M. Koivusalo and T. Vartiainen, *Rev. Environ. Health*, **12**, No. 2, 81-90 (1997).
7. J. R. Meier, *Mutat. Res.*, **196**, 211-245 (1988).
8. R. D. Morris, *Environ. Health Perspect.*, **103**, Suppl. 8, 225-231 (1995).