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Results of NTP-Sponsored Mouse Cytogenetic Studies on 1,3-Butadiene, Isoprene, and Chloroprene

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Studies were conducted to determine the cytotoxic and cytogenetic effects of 1,3-butadiene and two structural analogs, chloroprene and isoprene, in the bone marrow cells of B6C3F$_1$ mice exposed to the chemicals by inhalation. In one study, animals were exposed to 1,3-butadiene concentrations of 6.25, 62.5, or 625 ppm 6 hr/day on 10 exposure days and in the second study, to the same concentrations on weekdays for 13 weeks. Chloroprene and isoprene treatments involved 6 hr/day exposures on 12 exposure days at concentrations of 0, 12, 32, 80, and 200 ppm for chloroprene and 0, 438, 1750, and 7000 ppm for isoprene. In the 10-day study, 1,3-butadiene induced significant increases in sister chromatid exchange (SCE) at 6.25 ppm, micronuclei at 62.5 ppm, and chromosomal aberrations at 625 ppm. In the 13-week study, the frequency of micronucleated normochromatic erythrocytes in the peripheral blood was significantly elevated in all exposure groups including the 6.25-ppm group. Isoprene induced both SCE and micronuclei, whereas chloroprene gave negative results for all cytogenetic end points assessed in bone marrow cells.

Introduction

1,3-Butadiene is carcinogenic in both rats (*1*) and mice (*2*) following exposure by inhalation. Evidence of 1,3-butadiene's mutagenicity in the Ames Salmonella test was published in 1978 (*3*), and the induction of sister chromatid exchanges (SCE) and micronuclei in mouse bone marrow cells was reported in 1986 (*4–6*).

In conjunction with other studies being conducted by the National Toxicology Program on the toxic effects of 1,3-butadiene and the structurally similar compounds isoprene and chloroprene, experiments were conducted to evaluate the cytogenetic and cytotoxic effects of these compounds on the bone marrow cells of mice. Cytotoxicity was assessed as effects on the average generation time (AGT) and mitotic index (MI) in dividing bone marrow cells and on the frequency of polychromatic erythrocytes (PCE) in the peripheral blood. Cytogenetic effects in the bone marrow were determined using three end points: chromosomal aberrations, micronucleated (MN), polychromatic (PCE), or normochromatic (NCE) erythrocytes, and SCE.

These studies were carried out to: *a*) assess the individual and relative genetic toxicity of these three chemicals in mice; *b*) provide data for comparing the genetic toxicity and carcinogenicity of 1,3-butadiene; *c*) increase the data base on which the predictive value of short-term *in vivo* tests for carcinogens is being evaluated; and *d*) provide data to support an assessment of the potential carcinogenicity of isoprene and chloroprene.

Results of these NTP-sponsored studies have been published (*7–9*), and the present paper summarizes those study results.

Results and Discussion

The first studies conducted were on 1,3-butadiene (*7*). B6C3F$_1$ male mice, 12 per group, were exposed by inhalation to 0.0, 6.25, 62.5, or 625 ppm 6 hr/day on weekdays for 2 weeks (10 exposures). Both cytotoxic and cytogenetic effects were evident (Table 1).

Bone marrow cytoxicity was evident by virtue of the observations that *a*) the average generation time (AGT) was significantly extended at 62.5 ppm, *b*) percent PCE was elevated at 625 ppm, and *c*) the mitotic index (MI), although not significantly affected at any single dose point, was significantly depressed when analyzed by trend analysis.

All measures of cytogenetic effects showed dose-related responses. SCEs were significantly elevated at 6.25 ppm, MN-PCE at 62.5 ppm, and percent cells with chromosomal aberrations and MN-NCE at 625 ppm.

In a subsequent study of MN-PCE and MN-NCE in the peripheral blood (*8*), mice were exposed by inhalation to the same three concentrations previously described for 13 weeks. Peripheral blood samples were taken from 10 animals per group and 1000 PCE and 10,000 NCE were scored per animal. For PCE, results

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Table 1. Cytogenetic effects of 10 exposures to inhaled 1,3-butadiene in male B6C3F$_1$ mice (7).[a]

Exposure concentration, ppm	% Cells with chromosomal aberrations	SCE/ cell	MN-PCE/ 1000 PCE	MN-NCE/ 1000 NCE
0	2.3 ± 0.85[b]	5.51 ± 0.292	3.75 ± 0.592	2.67 ± 0.414
6.25	1.6 ± 0.36	6.49 ± 0.206*	5.50 ± 0.417	3.33 ± 0.497
62.5	3.3 ± 1.25	10.88 ± 0.398*	8.64 ± 0.789*	4.00 ± 0.618
625	14.0 ± 3.18*	35.09 ± 1.757*	30.00 ± 1.267*	7.17 ± 0.869*

[a]Abbreviations: SCE, sister chromatid exchange; MN-PCE, micronucleated-polychromatic erythrocytes; MN-NCE, micronucleated normochromatic erythrocytes.
[b]Mean ± SE.
*Significantly different from concurrent control.

Table 2. Frequencies of micronucleated normochromatic erythrocytes in the peripheral blood of B6C3F$_1$ mice exposed to 1,3-butadiene for 13 weeks (8).

Concentration, ppm	Male	Female
0	1.89 ± 0.18[a]	1.45 ± 0.16
6.25	2.56 ± 0.21*	1.90 ± 0.13*
62.5	4.97 ± 0.35*	4.04 ± 0.19*
625	16.63 ± 0.90*	12.15 ± 0.37*

[a]Mean per 1,000 cells ± SE, each based on 10 animals with at least 10,000 cells/animal.
*Significantly different from concurrent control.

of the 2-week study were confirmed with a significant increase in MN-PCE observed at 62.5 ppm (Table 2). However, in this study, the frequency of MN-NCE was significantly increased at the lowest concentration tested, i.e., 6.25 ppm in both males and females. The detection of a significant increase in MN-NCE at 6.25 ppm in this study can be attributed to the increased statistical power of the peripheral blood MN assessment where 10,000 NCE are scored per animal. In the bone marrow MN test, only 1000 cells per animal were scored, and although the frequency of MN-PCE increased with increasing exposure concentration, the magnitude of the effect at 6.25 ppm was not significant.

Isoprene was investigated in a 12-day exposure regimen using the same end points, route of exposure and species, strain, and sex of animals (*9*). Concentrations tested were 0, 438, 1750, and 7000 ppm. These are substantially higher concentrations than those used in the 1,3-butadiene tests described above, but they are similar to those used in the 1,3-butadiene 14-week prechronic mouse studies (*10*) where concentrations ranging from 625 to 8000 ppm were used.

Inhaled isoprene, 6 hr/day for 12 exposure days, resulted in significantly elevated frequencies of SCE, MN-PCE, and MN-NCE at all three exposure concentrations (Table 3). The percent cells with aberrations was elevated but not significantly. Bone marrow cytotoxicity was evident from an extended AGT at 7000 ppm and from a decrease in the percent PCE in the peripheral blood at all three concentrations.

Because the levels of effects observed were not dependent on the exposure concentrations, a second isoprene study was conducted using concentrations of 0, 70, 220, and 700 ppm. The purpose of this study was to determine if a dose effect curve was evident at concentrations below the apparent plateau that was observed in the first study.

In the second isoprene experiment, SCE showed a concentration-related effect and were significantly elevated at 700 and 220 ppm, but not at 70 ppm. The other cytogenetic end point scored, MN-PCE, was not elevated at 70 or 220 ppm but at 700 ppm they gave a value that was intermediate between the 438- and 1750-ppm

Table 3. Cytogenetic effects of 12 daily exposures to inhaled isoprene and chloroprene in the bone marrow cells of male B6C3F$_1$ mice (9).

	Concentration, ppm		% Cells with			
	Test I	Test II[a]	chromosomal aberrations[b]	SCE/cell[b]	% MN-PCE[b]	% MN-NCE[b]
Isoprene	0		2.25 ± 0.701[c]	4.40 ± 0.215	0.200	0.147
		0	1.25 ± 0.366	4.67 ± 0.345	0.390	0.423
		70	0.50 ± 0.327	4.89 ± 0.244	0.470	0.320
		220	0.75 ± 0.366	9.18 ± 0.772*	0.470	0.530
	438		3.75 ± 1.031	14.84 ± 1.909*	1.200*	0.520*
		700	1.00 ± 0.655	13.50 ± 1.226	1.390*	0.830*
	1750		4.50 ± 1.296	11.61 ± 0.261*	1.560*	0.640*
	7000		3.50 ± 0.732	13.98 ± 0.577*	1.693*	0.693*
Chloroprene	0		1.50 ± 0.327	5.06 ± 0.213	0.207	0.300
	12		1.25 ± 0.366	6.37 ± 0.827	0.314	0.257
	32		2.00 ± 0.756	4.61 ± 0.426	0.220	0.172
	80		0.85 ± 0.526	3.94 ± 0.329	0.260	0.280

[a]Results of test II with isoprene have not been previously published. The test was conducted as the previous study (*9*).
[b]Number of animals/number of cells scored per animal for aberrations = 8/50, SCE = 4/25, % MN-PCE = 15/1000, % MN-SCE = 15/1000.
[c]Mean ± SE of the mean.
*Significantly different from concurrent control.

value in the initial experiment. Thus, it appears that induced cytogenetic effects are dose dependent below about 500 ppm and that the levels of effects plateau at higher concentrations.

Chloroprene was investigated in similar experiments (*9*). Twelve-day exposures at 0, 12, 32, 80, and 200 ppm were administered to male B6C3F$_1$ male mice; 200 ppm was lethal to all exposed animals. The only significant effect observed in this study was an increase in the MI at the 80-ppm concentration (Table 3).

Conclusions

Inhaled 1,3-butadiene is genotoxic and cytotoxic in the mouse bone marrow, as demonstrated in the studies summarized here and in others (*11*). Induction of chromosomal effects are concentration dependent over a two order of magnitude range of exposures. The potent genetic toxicity of 1,3-butadiene in the mouse and the absence or low level of such effects in rats (*4,5*) are consistent with the relative carcinogenic effects in these two species (*11*).

Evidence of an increased frequency of micronucleated erythrocytes in mice exposed to concentrations of 1,3-butadiene below 10 ppm is a factor for consideration in setting human exposure limits.

Isoprene, like 1,3-butadiene, is a mouse bone marrow genotoxin and cytotoxin. Assuming the genetic toxicity of 1,3-butadiene is mechanistically related to its carcinogenic activity, the lower genetic toxicity of isoprene suggests that this analog of 1,3-butadiene will be tumorigenic in mice, although less so than 1,3-butadiene.

Chloroprene was more toxic to mice than the other two compounds, but it did not yield evidence of *in vivo* genetic toxicity in bone marrow cells. The *in vitro* mutagenicity of chloroprene (*12,13*) and its structural similarity to 1,3-butadiene suggest the need to investigate further the potential carcinogenic activity. The negative cytogenetic results reported here for chloroprene indicate that in inhalation-exposed male mice, there is no systemic exposure to the genotoxic form of the chemical. As an *in vitro* genotoxin with no apparent requirement for metabolic activation, one might predict that any carcinogenic activity exhibited by chloroprene would be evident at the sites of contact.

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