EVALUATION OF MICRONUCLEI IN THE PERIPHERAL BLOOD OF CD-1 MICE EXPOSED TO BENZENE

Phase I Study
2000 ppm - 5 Day Evaluation

Sponsored by
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Project: Evaluation of Micronuclei in the Peripheral Blood of CD-1 Mice Exposed to Benzene: Phase I Evaluation

Purpose

To assess the utility of evaluating micronuclei (MN) in the peripheral blood of male and female CD-1 mice exposed to benzene.

Protocol

At Hazleton Laboratories, CD-1 mice were exposed to benzene (2000 ppm, 6 hr per day, 5 days), and a blood sample was obtained on the morning of day 6. A typical blood smear was made and stained with Giemsa. The slides were mailed to Brookhaven National Laboratory and received on November 14, 1983, along with peripheral blood smears made from rats also exposed to benzene. At this time, it was impossible to identify the species origin of individual slides. On December 6, 1983, the code for the slides was received, the mouse slides were removed, destained with methanol: glacial acetic acid (5:1) for approximately 10 minutes, and randomized before staining with acridine orange (AO) at a concentration of 0.125 mg/ml in phosphate buffer, pH 7.4 (staining protocol - stain for 5 min, rinse in distilled water, remove excess stain by a further rinse in buffer for 10 min, and place a coverslip on the slide using buffer). The AO stained slides were analyzed by one technician with no knowledge of the experimental design of the study. For each slide, the number of MN in 1000 polychromatic erythrocytes (PCE) and in 1000 normochromatic erythrocytes (NCE), and the %PCE in 1000 red blood cells were evaluated at 800x in a Zeiss Standard microscope equipped with Epi-Fluorescence. The data were stored in a Vax 11/780 computer, the code broken, the animals grouped according to exposure conditions, and the data analyzed using nonparametric statistics with the BMDP Statistical Analysis Package.

Results

Table 1 lists the MN data on a per animal basis. Table 2 presents the group mean values and standard error between animals for MN per 1000 PCE, MN per 1000 NCE and %PCE. When the data are statistically analyzed (see Table 3), the following conclusions can be made.

1. There are no technical difficulties associated with destaining the Giemsa stained slides and evaluating the presence of MN using the more sensitive AO staining technique.

2. There were no significant differences in MN frequency in NCE or PCE, or in %PCE between concurrent controls and pretreatment controls.

3. Exposure to benzene (2000 ppm, 6 hr per day, 5 days) induced MN in the peripheral blood cells of CD-1 mice.

As expected, considering the short duration time of the exposure, MN frequencies were more elevated in the PCE population than in the NCE population. Also, consistent with other data, male mice tended to exhibit a greater genotoxic response than female mice.

4. Exposure to benzene induced a significant depression in the %PCE in CD-1 mice, the extent of the depression being equal in males and females.

Table 1. Individual Animal Micronuclei Frequencies (continued) 3.

Lab Acc. No. (8-29-83)	Lab Animal No.	Sex	% PCE*	MN/1000 NCE**	MN/1000 PCE***
2586	619	M	2.4	2	2
2587	625	M	7.5	3	2
2588	626	M	2.9	0	0
2589	629	M	2.4	1	1
2590	631	M	2.8	1	0
2591	686	F	4.2	1	1
2592	692	F	4.0	1	2
2593	693	F	5.0	0	0
2594	695	F	2.8	1	1
2595	703	F	1.6	0	8
2596	652	M	1.0	1	3
2597	657	M	0.7	3	3
2598	658	M	0.4	1	9
2599	663	M	4.2	0	3
2600	737	F	2.5	4	1
2601	740	F	0.3	0	0
2602	744	F	2.1	2	6
2603	746	F	1.6	0	2

* %PCE = % polychromatic erythrocyctes in 1000 red blood cells.
** MN/1000 NCE = Number of micronuclei cells in 1000 normochromatic erythrocytes.
*** MN/1000 PCE = Number of micronuclei in 1000 polychromatic erythrocytes.

Table 1. Individual Animal Micronuclei Frequencies (continued) 2.

Lab Acc. No. (8-28-83)	Lab Animal No.	Sex	% PCE*	MN/1000 NCE**	MN/1000 PCE***
2495	601	M	2.8	1	1
2496	605	M	1.1	0	3
2497	608	M	2.9	0	6
2499	616	M	2.1	0	1
2500	670	F	1.5	0	1
2501	673	F	0.8	1	0
2502	678	F	2.0	1	2
2503	679	F	3.3	2	1
2504	681	F	3.2	1	1
2505	633	M	5.2	2	1
2506	640	M	0.5	6	8
2507	642	M	1.3	4	2
2508	643	M	0.5	4	10
2509	647	M	0.3	5	3
2510	648	M	5.4	4	5
2511	716	F	1.0	3	8
2512	717	F	1.5	4	1
2513	721	F	0.6	1	9
2514	723	F	1.1	4	19
2515	724	F	2.3	2	4
2516	727	F	2.9	3	3

* %PCE = % polychromatic erythrocytes in 1000 red blood cells.
** MN/1000 NCE = Number of micronuclei cells in 1000 normochromatic erythrocytes.
*** MN/1000 PCE = Number of micronuclei in 1000 polychromatic erythrocytes.

Table 1. Individual Animal Micronuclei Frequencies

Lab Acc. No. (8-20-83)	Lab Animal No.	Sex	% PCE*	MN/1000 NCE**	MN/1000 PCE***
2061	04	M	2.7	0	2
2062	05	M	4.3	0	1
2063	11	M	2.6	0	1
2064	14	M	2.2	0	1
2064	08	M	-	-	-
2065	34	M	3.5	0	2
2066	36	M	2.1	0	0
2067	37	M	2.9	1	1
2068	44	M	2.7	3	1
2069	56	M	4.7	1	1
2070	62	M	2.3	1	1
2071	08	F	2.0	0	0
2072	09	F	2.4	4	1
2073	12	F	3.0	3	0
2074	14	F	2.0	1	2
2075	21	F	1.8	1	0
2076	26	F	2.2	1	1
2077	28	F	3.5	1	4
2078	30	F	4.4	2	3
2079	40	F	2.2	8	1
2080	43	F	3.4	1	1

* %PCE = % polychromatic erythrocyctes in 1000 red blood cells.
** MN/1000 NCE = Number of micronuclei cells in 1000 normochromatic erythrocytes.
*** MN/1000 PCE = Number of micronuclei in 1000 polychromatic erythrocytes.

Table 2. Micronuclei Frequencies in the Peripheral Blood Cells of CD-1 Mice Exposed to Benzene.

Exposure Group	Sex	Number Animals	MN/1000 NCE*	MN/1000 PCE**	% PCE+
Pretreatment	Male	10	0.60 ± 0.31	1.10 ± 0.18	3.0 ± 0.3
Controls	Female	10	2.20 ± 0.74	1.00 ± 0.30	2.7 ± 0.3
	M + F	20	1.40 ± 0.43	1.05 ± 0.17	2.8 ± 0.2
Concurrent	Male	10	0.80 ± 0.33	1.70 ± 0.56	2.8 ± 0.6
Controls	Female	10	1.00 ± 0.21	1.70 ± 0.73	3.2 ± 0.5
	M + F	20	0.90 ± 0.19	1.70 ± 0.45	3.0 ± 0.4
Benzene	Male	10	3.20 ± 0.61	4.80 ± 0.96	1.5 ± 0.6
Treated	Female	10	2.30 ± 0.50	5.30 ± 1.80	1.7 ± 0.3
	M + F	20	2.75 ± 0.40	5.05 ± 1.00	1.6 ± 0.3

*Micronuclei per 1000 normochromatic erythrocytes
**Micronuclei per 1000 polychromatic erythrocytes
+per cent polychromatic erythrocytes in 1000 red blood cells

Table 3. Statistical Analysis of MN Frequencies and %PCE in the Peripheral Blood Cells of CD-1 Mice Exposed to Benzene

A. Micronuclei Frequencies

		Pretreatment Controls			Concurrent Controls			Benzene Treated		
		M	F	M+F	M	F	M+F	M	F	M+F
		PCE "P" VALUES								
Pretreatment Controls	M	-			.56			.00*		
	F		-			.54			.02*	
	M+F			-			.42			.00*
Concurrent Controls	M	.61			-			.00*		
	F		.21			-			.06	
	M+F			.71			-			.00*
Benzene Treated	M	.00*			.01*			-		
	F		.53			.05*			-	
	M+F			.01*			.00*			-
		NCE "P" VALUES								

P values are calculated from the Kruskall-Wallis One-Way ANOVA using Chi-Square Distribution with one degree of freedom.

* p value significantly different at less than or equal to 0.05.

Table 3. Statistical Analysis of MN Frequencies and %PCE in the Peripheral Blood Cells of CD-1 Mice Exposed to Benzene

B. %PCE Determinations

		Pretreatment Controls			Concurrent Controls			Benzene Treated		
		M	F	M+F	M	F	M+F	M	F	M+F
Pretreatment Controls	M	-								
	F		-							
	M+F			-						
Concurrent Controls	M	.52								
	F		.45							
	M+F			.88						
Benzene Treated	M	.02*			.02*					
	F		.02*			.01*				
	M+F			.00*			.00*			

P values are calculated from the Kruskall-Wallis One-Way ANOVA using Chi-Square Distribution with one degree of freedom.

* p value significantly different at less than or equal to 0.05.