Short Communication

MICRONUCLEUS TESTS IN MICE
ON FOUR CHROME-CONTAIN-
ING PIGMENTS

4種のクロム含有色素のマウス小核試験

Key words: chrome-containing pigment; chrome-yellow; molybdenum-red; zinc potassium chromate; zinc tetroxychromate; micronucleus test

Despite the wide use of industrial chrome-containing pigments, limited data concerning mutagenic or clastogenic activity are available for these compounds.¹⁻³⁾ Although lead chromate is a potent carcinogen in rat,⁴⁾ equivocal results have been reported in various *in vitro* mutation assay systems.⁵⁻⁷⁾ Four chrome-containing pigments which are in current production and use in Japan were studied for clastogenic activity. The insoluble pigments including chrome-yellow (lead chromate) and molybdenum-red (lead chromate mixed with lead molybdate) and sodium dichromate, a highly soluble chromate were tested by the micronucleus test in mouse bone marrow.

Materials and methods. Chemicals are listed in Table 1. Chrome-yellow (CY), zinc potassium chromate (ZPC) and zinc tetroxychromate (ZTO) were kindly provided by Tohoganryo Co. Ltd., Tokyo, Japan, and molybdenum-red (MR) by Dainihon-seika Kogyo Co. Ltd., Tokyo, Japan. Sodium dichromate was analytical-grade reagent (Wako).

Nine-week-old female ICR/Jcl mice were used. They were fed with basal diet (pellet CE-2, Clea Japan), and water was given *ad libitum*. Four mice per group were injected intraperitoneally with 25, 50 and 100 mg/kg bw. of CY and MR, or 12.5, 25, 50 and 100 mg/kg bw. of ZPC and ZTO. The majority of animals which received 100 mg/kg bw. of ZPC or ZTO were dead within 24 h after the injection, but lower levels could be tolerated. Sodium dichromate was injected at doses of 12.5, 25 and 50 mg/kg bw. For each chemical, four additional mice were given a total of twice the maximum tolerated dose by two single dose injections separated by a 24-h interval.

Because uniform suspensions of CY, MR, ZPC and ZTO could not be prepared in physiological saline, these compounds were suspended in olive oil; solvent treated animals served as a negative

Table 1. Chemical formula and amount of water-soluble material of chromium compounds.

Compound	Chemical formula ¹	Water-soluble material (%)
Chrome-Yellow (CY)	PbCrO ₄	< 1
Molybdenum-Red (MR)	PbCrO ₄ ·nPbMoO ₄ ·mPbSO ₄ ·xAl(OH) ₃	< 1
Zinc potassium chromate (ZPC)	K ₂ O·4ZnO·4CrO ₃ ·3H ₂ O	< 6 - 8
Zinc tetroxychromate (ZTO)	ZnCrO ₄ ·4Zn(OH) ₂	< 1
Sodium dichromate ²	Na ₂ Cr ₂ O ₇	Very soluble (2380 g/l at 0°C) ³

¹: As reported by producers.

²: Sodium dichromate was analytical grade reagent.

³: Solubility.

control. Sodium dichromate was dissolved in physiological saline. Animals injected intraperitoneally with 2.5 mg/kg bw. of mitomycin C served as positive control.

The animals were killed by cervical dislocation 24 h after the single injection, or 6 h after the second injection in the multiple treatments, and the bone marrow smear preparations were made according to Schmid's method.⁸⁾ One thousand polychromatic erythrocytes (PCEs) per mouse were examined and the number containing micronuclei was scored. Statistical analysis was carried out by the binominal distribution test.⁹⁾

Results. The results obtained with the micronucleus test are shown in Table 2. No significant induction of micronuclei was observed in bone marrow erythrocytes of mice treated with any of the pigments; CY, MR, ZPC and ZTO. In contrast, the animals receiving single/double injection(s) of sodium dichromate showed a significantly increased number of micronucleated polychromatic erythrocytes (MNPCEs). The compounds, ZPC and ZTO, showed cytotoxicity at the highest dose levels as evidenced by the significant reduction in PCEs frequency (Table 2).

Discussion. It is accepted that the genotoxic actions of chromium compounds are closely associated with the oxidative state of chromium and the solubility of the compounds. In the present study, the very soluble hexavalent chromate, sodium dichromate was prove to be clastogenic. In contrast, all the four pigments which are water-insoluble or only slightly soluble, hexavalent chromates yielded negative results in the micronucleus test.

The formation of micronuclei can be expected only if a sufficient amount of the active mutagenic form of the test compounds reaches the target

Table 2. Frequencies of micronucleated polychromatic erythrocytes in mouse bone marrow after treatment with chrome-containing pigments or sodium dichromate.

Chemical	Intraperitoneal doses (mg/kg)	No. mice examined	No. PCEs observed	Freq. of PCEs ^a		MNPCEs ^b	
				Number	Freq. (%)	Number	Freq. (%)
Chrome-Yellow (CY)	0	4	4000	58 ± 4 ^d	4	1.0 ± 0.8 ^d	
	25	4	4000	55 ± 4	2	0.5 ± 0.6	
	50	4	4000	56 ± 2	5	1.3 ± 1.0	
	100	4	4000	56 ± 2	2	0.5 ± 0.6	
Polydenum-Red (MR)	0	4	4000	52 ± 7	4	1.0 ± 0.6	
	25	4	4000	59 ± 3	3	0.8 ± 1.0	
	50	4	4000	59 ± 2	3	0.8 ± 0.5	
	100	4	4000	56 ± 4	5	1.3 ± 1.0	
Zinc potassium chromate (ZPC)	0	4	4000	53 ± 3	3	0.8 ± 0.5	
	12.5	4	4000	53 ± 4	4	1.0 ± 0.8	
	25	4	4000	55 ± 5	5	1.3 ± 0.5	
	50	4	4000	49 ± 2	4	1.0 ± 0.8	
Zinc tetraoxy-chromate (ZTO)	0	4	4000	45 ± 4 ^e	5	1.3 ± 0.5	
	12.5	4	4000	39 ± 1 ^f	7	1.8 ± 0.5	
	25	4	4000	33 ± 6 ^f	6	1.5 ± 0.7	
	50	4	4000	57 ± 4	5	1.3 ± 1.0	
Sodium dichromate	0	4	4000	55 ± 2	4	1.0 ± 0.8	
	12.5	4	4000	53 ± 2	3	0.8 ± 1.0	
	25	4	4000	48 ± 6 ^e	5	1.3 ± 1.3	
	50	4	4000	42 ± 6 ^f	7	1.8 ± 1.0	
Mitomycin C	0	4	4000	52 ± 3	3	0.8 ± 0.5	
	12.5	4	4000	50 ± 3	5	1.3 ± 1.0	
	25	4	4000	44 ± 4	8	2.0 ± 0.8	
	50	4	4000	39 ± 4 ^f	27	6.8 ± 1.7 ^f	
	50 X 2	4	4000	38 ± 6 ^f	30	7.5 ± 2.0 ^f	
	2.5	4	4000	36 ± 5 ^f	214	53.5 ± 6.4 ^f	

^a: Polychromatic erythrocytes

^b: Micronucleated polychromatic erythrocytes

^c: Per total 1000 erythrocytes

^d: Mean ± S.D.

^e: p < 0.05

^f: p < 0.01

organ, the bone marrow. Therefore, it has been generally recommended that doses approaching LD₅₀ for 7-day survival should be applied in the micronucleus test.¹⁰⁾

From this point of view, the negative results obtained with ZPC and ZTO are acceptable because the animals were injected with dose sufficient to show toxic effects in some animals as shown by the marked reduction in PCEs frequency used as an index of cytotoxicity (Table 2). On the other hand, the negative results in the micronucleus test of CY and MR cannot be accepted as definitive, because the treatment with either chemicals did not show evidence for absorption and transport of these compounds to bone marrow, as evidenced by a lack of reduction in PCEs frequency (Table 2).

Workers exposed to paint products are in fact exposed to complex mixtures of various chrome-containing compounds. The relative insolubility of these compounds in aqueous solutions means they are difficult to test by a standard *in vivo* genotoxicity assay such as micronucleus test. Their lack of effect in this assay is indicative more of methodological shortcomings than of lack of genotoxicity.

Aqueous-insoluble pigments may pose a real and significant genotoxic hazard. The testing of such compounds requires correct interpretation of negative findings and development of suitable modifications to current tests to assess genotoxicity findings unequivocally. In fact, CY and MR have been found to be effective for inducing chromosomal aberrations and sister chromatid exchange in cultured human lymphocytes.⁷⁾ *In vitro* micronucleus assay, whereby cells can be placed in direct contact with a high dose of such chemicals, may provide additional information on the cytogenetic effect of such insoluble chrome-containing pigments.

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