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Short Communication

LACK OF MUTAGENIC EFFECT OF VINYL CHLORIDE MONOMER IN THE MAMMALIAN SPOT TEST

SÁNDOR PETER^{1,2,*} and GYÖRGY UNGVÁRY¹

¹ State Institute of Hygiene, Laboratory of Human Genetics, ² Chinom Chemical and Pharmaceutical Works Ltd., and ³ State Institute of Occupational Health, Department of Experimental Pathology, Budapest (Hungary)

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Evaluation of genetic hazards of exposure to vinyl chloride monomer (VCM) is one of the most serious current problems in occupational medicine. Numerous tests have been done to check the mutagenicity of VCM. Many of these were carried out on *sub-mammalian* systems [2-4,7,8,15,22,25,26,29-31], others on *mammals in vivo* [1,12,27,29,33], and on *human lymphocytes in vitro* [5,9,12-14,16-18,20,21,27,28,32]. The sub-mammalian examinations shed light on the importance of the metabolism of VCM in the relation to the mutagenicity [2-4,7,15,19,22,29-31]. Chromosome analysis of human lymphocytes demonstrated dose-dependent [12,17,20] and time-dependent [32] mutagenic effects, showing the importance of the chronic exposure to VCM [9,13,14,16,27,28] as opposed to the acute administration [8]. Inducibility of recessive gene mutations have been studied in *Drosophila melanogaster*, too. These positive findings [23,24,29,33] have shown that it might be possible to induce somatic gene mutation in mammals *in vivo*, too, an assumption that we wanted to verify by the mammalian spot test. The method we used was described by Fahrng [10].

Female mice of the inbred C57BL/6JHan (*a/a*; wild-type) were mated to the Han rotated-bred males of the T-stock strain (*a/a*, non-agouti; *b/b*, brown; *c^{eh}p/c^{eh}p*, chinchilla, and pinked eye dilution; *dse/dse*, dilute and short ear; *s/s*, piebald spotting). On the morning of the 10th day after the appearance of the vaginal plugs, the C57BL/6JHan females were exposed to 12 000 mg/m³ (4600 ppm) atmospheric concentration of VCM for 5 h. The VCM concentration in the inhalation chamber was determined by a type 5840-A Hewlett Packard digital gas chromatograph. The F₁ offspring were examined at 3-5 weeks post-partum for the appearance of mosaic coat colours. To check the ability to induce coloured spots in F₁ hybrids of our parent mice, we examined the mutagenic effects of cyclophosphamide (Jenapharm, GDR) (CP), too. This experiment served as positive control. The statistical evaluation of the data was done by the chi-square test.

* Present address: Department of Behaviour Genetics, L. Eötvös University, H-2131 God, Javorka S. u. 14 (Hungary).

TABLE 1

SURVIVAL AND SPOT INCIDENCE IN OFFSPRING OF C57 BL/6JIL^a X T-STOCK FOLLOWING TREATMENT OF VCM AND CP ON THE 10th DAY OF PREGNANCY

Agents	Dose	Females		Offspring									
		Treated (n)	Without litter (n)	Born (n)	Average litter size (n)	Survived to obser- vation (n)	Average litter size (n)	Without spots		With colour spots			
										White		Brownish	
								(n)	(%)	(n)	(%)	(n)	(%)
VCM ^a	12 000 mg/m ³	41	2	216	5.1	216	5.1	210	97.2	6	2.8	0	0
CP ^b	10.0 mg/kg	31	3	122	4.4	126	3.8	96	90.6	7	6.6	3	2.8
contr.	0	51	3	252	5.3	250	5.2	247	98.8	3	1.2	0	0

^a Inhalation.^b s.c.

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The data and the results of the experiment are summarized in Table 1. Altogether, 216 F_1 offspring of 44 treated parents were observed in the VCM-exposure group. There was no difference in the average litter size at birth or at the time of observation 3–5 weeks after birth. No offspring with abnormal morphology could be found among the animals. Mid-ventral white spots occurred at a frequency of 2.8%, which does not significantly differ from the relative control value ($p > 0.05$). Treatment with 10 mg CP/kg did not induce a marked difference in the number of offspring in comparison with the control or the VCM-treated group. There were differences, however, in the number of white and coloured spots; both were significant. All coloured spots were brownish. The distribution of these spots was random among litters.

No data on the mutagenic effect of VCM on genes, studied on mammals *in vivo*, have yet been published. Studies on sub-mammalian systems show that VCM can cause *point mutation*, especially base-pair substitution after metabolic activation, *in vitro* [2–4,15,30] and *in vivo* [9], although VCM itself does not produce such effect without the presence of liver microsomes [8]. Therefore it was assumed that VCM would exert a mutagenic effect in the somatic coat-colour (spot) test. The dose used in our test was based on the results obtained in previous acute experiments. This toxicity study showed that this dose was just tolerable, but not yet toxic for the animals. The observed survival rate of the offspring in our work confirmed this conclusion. This work provides no evidence that VCM induces somatic gene mutation. The experiment with CP showed that this lack of inducibility of coat-colour mosaic in F_1 could not have been due to some inherent deficiency in the test system used.

Nevertheless there is no evidence that either the application of fractionated doses during the 10th day (pc) or single doses of VCM, and/or continual exposure on the 8–10th days would not have led to a positive result. However, there are no data about chemical agents, which, though they have produced somatic-colour mosaics on the 8th or 9th day, fail to do so on the 10th. The reason for this may be that there are only two types of real effect chemical agents have in the mammalian spot test: (1) an agent can produce a maximal response after treatment on one day between the 8th and 10th days (pc), e.g. ethyl methanesulphonate (EMS) [11]; (2) the other type of effect shows equal response at any time from the 8th to 10th days in the spot test system. This type of effect can be seen with benzo[a]pyrene (B[a]P) [6]. Naturally, a negative result may be regarded as the 3rd type of effect. Because of the negative result on the 10th day, VCM does not have any of the real effects, and the results provide no evidence that VCM causes gene mutation.

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