The Protective Role of Thiola against the Genotoxic Effect of Potassium Dichromate in Mice *in vivo*

Maha A. Fahmy^{1,*}, Soheir Abdel-Kawy² and Entesar E. S. Hassan¹

¹ Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

² Faculty of Science-Al-Azhar University, Cairo, Egypt

Accepted November 13, 2000

Summary The genotoxic effect of hexavalent potassium dichromate ($K_2Cr_2O_7$) was investigated in mice *in vivo* using sister chromatid exchange (SCE) and chromosomal aberration analysis. $K_2Cr_2O_7$ induced a significant increase in the frequency of SCE's after intraperitoneal (i.p.) treatment with the doses 6, 12, 24 mg kg^{-1} b.wt. which correspond to 1/8, 1/4 and 1/2 of the experimental LD_{50} . The lowest tested dose 3 mg kg^{-1} b.wt. had no effect with respect to SCE's and its effect reached 6.57 ± 0.36 /cell compared with 5.80 ± 0.55 /cell for the control. The frequency of SCE's reached 9.03 ± 0.20 after treatment with the highest tested dose of $K_2Cr_2O_7$, a value which is less than that induced by mitomycin C (13.10 ± 0.40) as the positive control. Thiola at the concentrations of 20 and 50 mg kg^{-1} b.wt. had moderate but non-significant effect for minimizing the frequency of SCE's induced by different doses of $K_2Cr_2O_7$.

With respect to chromosomal aberrations, all the tested concentrations of $K_2Cr_2O_7$ (3, 6, 12, 24 mg kg^{-1} b.wt.) induced a significant increase in the percentage of chromosomal aberrations in mouse bone marrow as well as in mouse spermatocytes 24 h after single i.p. treatment. The incidence of chromosomal damage increased significantly with increasing the dose. However mitomycin C induced higher effect. The results also show that the pretreatment with thiola at the dose of 50 mg kg^{-1} b.wt. significantly reduced the percentage of chromosomal aberrations induced by $K_2Cr_2O_7$ in all the treatment groups and the results confirm the protective role of thiola which has been proved previously against the genotoxicity of some mutagens.

The contamination of our environment with metal compounds has expanded to include the general public health. Metal mutagenesis has only been little studied, although many of them are reported to be carcinogenic (IARC 1990, Singh *et al.* 1999, Carlisle *et al.* 2000).

Mutagenesis by chromium is of potential interest in view of human exposure to this metal. The main uses of chromium salts are metallurgical, refractory and chemical industries. The high coloured salts of chromium are used as pigments and as mordants in textile industry, in tanning, in photographic emulsions, as catalysts for organic and inorganic reactions and in ceramics. Chromium is also used in explosives (Leonard and Lauwerys 1980).

The actual health problems due to chromium salts seems to be restricted to hexavalent chromium Cr^{6+} . This is probably because hexavalent form can cross cell membrane easily *via* sulfate transport system and be reduced intracellularly to trivalent chromium which is the ultimate toxic agent capable of binding to cellular components (Biedermann and Landolph 1990). Cr^{6+} compounds induce point mutation in bacteria (Bianchi *et al.* 1983), and yeasts (Bonatti *et al.* 1976), morphological infidelity of DNA replication *in vitro* (Bianchi *et al.* 1983), morphological transformation in hamster embryonic cells (Tsuda and Kato 1977) and chromosomal aberrations in cultured human lymphocytes *in vitro* (Alain *et al.* 1997). However there are few reports concerned with its mammalian *in vivo* mutagenicity (Knudsen 1980, Itoh and Shimada 1998).

Among Cr^{6+} compounds, potassium dichromate ($K_2Cr_2O_7$) is strong cytotoxic agent in mam-

* Corresponding author.

malian cell cultures, differentially inhibiting nucleic acid and protein synthesis and affecting the uptake of nucleosides and amino acids through the plasma membrane (Levis *et al.* 1978a, b). $K_2Cr_2O_7$ also interferes with the mitotic process, giving rise to different kinds of mitotic blockage and cytological alterations (Majone 1977).

In the present article we describe the induction of sister chromatid exchanges (SCE's) and chromosomal aberrations in both somatic and germ cells of male mice *in vivo* after single i.p. treatment with different concentrations of $K_2Cr_2O_7$. The protective effect of 2-mercaptopropionylglycine (thiola) was also studied. Thiola is a sulfhydryl compound widely used as detoxicating agent and also has a variety of pharmacological activities (Ezzat *et al.* 1982). Thiola was found to possess significant reduction in the frequency of chromosomal aberrations induced by radiation (Uma Devi and Prasanna 1990) and some chemical mutagens (Galdean *et al.* 1986, Schunck *et al.* 1998). Therefore, the protective role of thiola was studied in a trial to minimize the genotoxicity of $K_2Cr_2O_7$.

Materials and methods

Animals

Mature male white Swiss mice aged 9–12 weeks and weighing 25–30 g were used in all experiments. The tested animals were obtained from a closed random-bred colony at the National Research Centre, maintained under controlled conditions of temperature and humidity and receiving food and water *ad libitum*.

Chemicals

Potassium dichromate (99.5%) was purchased from S.D. FINE. CHEM. Ltd. Egypt.

Thiola: N-(2-mercaptopropionylglycine) was purchased from Sigma Chemical Co., St. Lewis, Mo. U.S.A.

Treatment and cytological preparations

Sister chromatid exchange: Four doses of $K_2Cr_2O_7$ (dissolved in distilled water) were tested i.p. for the induction of SCE's in mouse bone-marrow cells. The doses were 3, 6, 12, 24 mg kg⁻¹ b.wt. which correspond to 1/16, 1/8, 1/4, 1/2 LD₅₀. The LD₅₀ of $K_2Cr_2O_7$ was determined experimentally. The experimental procedure was conducted according to Madrigal-Bujaidar *et al.* (1998). A subcutaneous implantation of 5-bromodeoxyuridine (BrdU, Sigma) tablet weighing 55 ± 5 mg was initially made in each mouse and 30 min later the test compound was administered by an i.p. injection. After 21 h the animals received an i.p. injection of colchicine (0.6 mg kg⁻¹ b.wt.) and 2 h later, the bone-marrow of both femur were obtained in KCL 0.075 M at 37°C keeping the cells 20 min at the same temperature, then the fixation process with methanol–acetic acid (3 : 1) was done changing the solution at least twice. Metaphase spreads were air-drying prepared. Chromatid differentiation was achieved with the fluorescence plus Giemsa method of Goto *et al.* (1978) with the use of black lights. About 40 metaphase spreads per animal were examined microscopically for SCE's.

The protective effect of thiola was screened by using 2 concentrations of thiola 20 and 50 mg kg⁻¹ b.wt. which are far below its toxic dose (2100 mg/kg) as reported by Sugahara and Srivastava (1976). Each concentration was tested in 3 groups of mice given $K_2Cr_2O_7$ at concentrations of 6, 12, 24 mg kg⁻¹ b.wt. Thiola was injected i.p. 2 h prior to the administration of $K_2Cr_2O_7$.

Chromosomal aberration: For bone marrow or spermatocytes, groups of 5 animals were injected i.p. with a single dose of $K_2Cr_2O_7$ at the doses of 3, 6, 12, 24 mg kg⁻¹ b.wt. Mice were killed 24 h post treatment. Another groups of mice were i.p. injected with thiola 50 mg kg⁻¹ b.wt. 2 h prior to the administration of $K_2Cr_2O_7$ at the doses of 6, 12, 24 mg kg⁻¹ b.wt. For bone-marrow and spermatocyte metaphase preparations, air-dried techniques were made according to Yosida and Amano (1965) and Evans *et al.* (1964) respectively. Slides were stained with phosphate-buffered Giemsa

7%. At least 75–100 well spread metaphases were analyzed per each animal scoring different types of chromosomal aberrations.

For all the above experiments, a control group of non-treated mice was run alongside the test material in addition to the positive control group treated i.p. with 1 mg kg^{-1} b.wt. mitomycin C.

Statistical analysis

In all experiments the resulting data were compared statistically using Fisher's least significant differences and Duncan's multiple range test. The significance of the incidence of abnormalities in experimental versus control data was calculated using the *t*-test.

Results and discussion

The ability of $\text{K}_2\text{Cr}_2\text{O}_7$ to induce SCE's in mice bone marrow cells was investigated i.p. at different dose levels 3, 6, 12, 24 mg kg^{-1} b.wt. (Table 1, Fig. 1a–c). The doses 6, 12, 24 mg kg^{-1} b.wt. induced a significant increase in the frequency of SCE's over that of the control with a dose-related relationship. Such frequency of SCE's reached 7.90 ± 0.33 , 8.40 ± 0.25 , 9.03 ± 0.20 per cell after treatment with the 3 doses respectively compared with 5.80 ± 0.55 per cell for the control.

The frequency of SCE's induced after treatment with $\text{K}_2\text{Cr}_2\text{O}_7$ was lower than that induced by mitomycin C ($13.10 \pm 0.40/\text{cell}$) which is well known to be strong SCE's inducing agent (Russo *et al.* 1993) and was included as a positive control. The results in the present work are in agreement with the previous findings of Majone and Levis (1979) and Bianchi *et al.* (1983) who reported that $\text{K}_2\text{Cr}_2\text{O}_7$ induced a dose related increase in the frequency of SCE's in Chinese hamster cells (CHO) and in other mammalian cell systems *in vitro*. The same authors reported that mitomycin C induced higher effect. A linear increase in the frequency of SCE's was also reported by Ohno *et al.* (1982) concerning the effect of chromium compounds in Don Chinese hamster cells.

Thiola is one of the most potentially active radioprotector and is widely used as detoxicating agent (Ezzat *et al.* 1982). Thiola was successfully reduced the frequencies of SCE's induced by neocarzinostatin in Chinese hamster ovary (CHO) cell (Schunck *et al.* 1998). In the present work 2 different doses of thiola 20 and 50 mg kg^{-1} b.wt. were tested i.p. to minimize the effect of $\text{K}_2\text{Cr}_2\text{O}_7$ on SCE's. The results show that thiola did not induce SCE's of its own at the tested concentrations and its effect was statistically non significant compared with the control. The administration of thiola 2 h prior to $\text{K}_2\text{Cr}_2\text{O}_7$ moderately minimized the induced $\text{K}_2\text{Cr}_2\text{O}_7$ SCE's with statistical non-significant result as compared with that groups treated with $\text{K}_2\text{Cr}_2\text{O}_7$ alone (Table 1).

Single i.p. treatment with $\text{K}_2\text{Cr}_2\text{O}_7$ at the doses of 3, 6, 12, 24 mg kg^{-1} b.wt. induced also significant percentage of chromosomal aberrations in mouse bone-marrow cells (Table 2, Fig. 1d–f). Such increase was found to be dose-dependent. It reached 14.67 ± 0.40 after treatment with the highest tested dose compared with 4.80 ± 0.33 for the control. The value is less than that induced after i.p. treatment with mitomycin C (28.30 ± 0.26) as the positive control. The main types of aberrations observed were gaps, isochromatid breaks, fragments and deletions. Few cells with translocations were observed. Numerical chromosomal aberrations in the form of endomitosis (C-mitosis) were observed in all treated groups suggesting an effect on the mitotic spindle (Benjamin and Stephen 1996). These results are outlined with the previous data in cultured mammalian cells which showed that $\text{K}_2\text{Cr}_2\text{O}_7$ behaves as a powerful clastogenic agent, producing a significant increase in chromosomal aberrations including gaps, breaks and exchanges when added to hamster embryonic cells (Tsuda and Kato 1976, 1977), to a cultured Chinese hamster cell line (Newbold *et al.* 1979), to FM₃A cells from a C₃H mouse carcinoma (Umeda and Nishimura 1979) to human lymphocyte cultures (Nakamuro *et al.* 1978). Majone (1977) reports that this compound induces mitotic inhibition as well as perturbation in mitotic spindles in human cells (HEP line).

Moreover $\text{K}_2\text{Cr}_2\text{O}_7$ showed similar dose effect relationship in micronucleus assay using the

Table 1. Frequency of sister chromatid exchanges (SCE's) in mouse bone-marrow cells after i.p. treatment with potassium dichromate and thiola

Treatment and doses		No. of mice	No. of examined metaphases with differentiation	SCE's No.	SCE's/Cell Mean $\pm$ S.E.
I.	Control (non-treated)	5	200	1160	5.80 $\pm$ 0.55
II.	Potassium dichromate (mg kg ⁻¹ b.wt.)				
	3	5	200	1314	6.57 $\pm$ 0.36
	6	5	200	1580	7.90 $\pm$ 0.33*
	12	5	200	1680	8.40 $\pm$ 0.25**
	24	5	200	1806	9.03 $\pm$ 0.20**
III.	Thiola (50 mg kg ⁻¹ b.wt.)	5	200	1220	6.10 $\pm$ 0.13
IV.	Potassium dichromate (mg kg ⁻¹ b.wt.) + Thiola (50 mg kg ⁻¹ b.wt.)				
	6	5	200	1500	7.50 $\pm$ 0.22*
	12	5	200	1536	7.68 $\pm$ 0.27*
	24	5	200	1676	8.38 $\pm$ 0.38**
V.	Thiola 20 mg kg ⁻¹ b.wt.	5	200	1200	6.00 $\pm$ 0.28
VI.	Potassium dichromate (mg kg ⁻¹ b.wt.) + Thiola (20 mg kg ⁻¹ b.wt.)				
	6	5	200	1396	6.98 $\pm$ 0.20*
	12	5	200	1520	7.60 $\pm$ 0.60*
	24	5	200	1630	8.15 $\pm$ 0.14**
VII.	Mitomycin C (Positive control) 1 mg kg ⁻¹ b.wt.	5	200	2620	13.10 $\pm$ 0.40***

* Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level (*t*-test).

Fig. 1. Metaphases from mouse bone-marrow showing. a-c) SCE's induced after treatment with different doses of $K_2Cr_2O_7$, d) chromatid break, e) fragment, f) gap and break. Metaphases from mouse spermatocytes treated with $K_2Cr_2O_7$ showing. g) X-Y univalent, h) autosomal univalent, i) break.

Table 2. Frequency of chromosomal aberrations in mouse bone-marrow cells induced after single i.p. treatment with $K_2Cr_2O_7$ and thiola

Treatment and doses	No. of examined metaphases (n) H ^o	No. of abnormal metaphases	No. and % of metaphases with different types of chromosomal aberrations ^a										Mean % metaphases with chromosomal aberr. ± S.E.	
			Chromat. gap (%)	Chromos. gap (%)	Break (%)	Frag. (%)	M.A. (%)	Del. (%)	RT (%)	Tetrap. (%)	Endomit. (%)	including gaps	excluding gaps	
I. Control (non-treated)	375	18	8 (2.13)	—	2 (0.63)	6 (1.60)	—	2 (0.53)	—	—	—	4.80±0.33	2.66±0.4	
II. Potassium dichromate (mg kg ⁻¹ b.wt.)														
3	375	25	9 (2.40)	—	3 (0.80)	3 (0.80)	—	7 (1.87)	—	—	3 (0.80)	6.67±0.73*	4.26±0.65	
6	375	40	13 (3.47)	—	9 (2.40)	3 (0.80)	—	8 (2.13)	—	—	4 (1.07)	10.67±0.73**	7.20±0.70**	
12	375	46	13 (3.47)	1 (0.27)	6 (1.60)	5 (1.33)	—	9 (2.40)	—	1 (0.27)	5 (1.33)	12.27±0.50**	8.53±0.68**	
24	375	55	14 (3.73)	—	7 (1.87)	8 (2.13)	—	14 (3.73)	3 (0.80)	—	6 (1.60)	14.67±0.40**	10.93±0.50**	
III. Thiola (50 mg kg ⁻¹ b.wt.)	375	17	7 (1.87)	—	—	5 (1.33)	—	5 (1.33)	—	—	—	4.53±0.50	2.66±0.40	
IV. Potassium dichromate (mg kg ⁻¹ b.wt.)														
+Thiola (50 mg kg ⁻¹ b.wt.)														
6 +Thiola	375	25	12 (3.20)	—	6 (1.60)	2 (0.53)	—	4 (1.07)	—	—	1 (0.27)	6.67±0.60*	3.50±0.33	
12 +Thiola	375	30	10 (2.66)	—	6 (1.60)	6 (1.65)	—	4 (1.07)	1 (0.27)	—	2 (0.53)	8.00±0.40**	5.33±0.40**	
24 +Thiola	375	33	11 (2.93)	—	9 (2.40)	7 (1.87)	—	2 (0.53)	—	—	3 (0.80)	8.80±0.33**	5.87±0.53**	
VII. Mitomycin C 1 mg kg ⁻¹ b.wt. (Positive control)	375	106	21 (5.60)	7 (1.87)	16 (4.30)	17 (4.50)	19 (5.07)	13 (3.50)	9 (2.40)	4 (1.07)	—	28.30±0.26	20.8±1.00***	

(n)=Number of animals per each treatment group=5, H^o =Harvest time after treatment=24 h, ^a Chromat. gap=Chromatid gap, Chromos. gap=Chromosome gap, Frag.=Fragment, M.A.=Metaphases containing more than one type of chromosomal aberrations, Del.=Deletion, RT=Robertsonian translocation, Tetrap.=Tetraploid, Endomit.=Endomitosis * Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level (t-test).

Table 3. Frequency of chromosomal aberrations in mouse spermatocytes induced after single i.p. treatment with $K_2Cr_2O_7$ and thiola

Treatment and doses	No. of examined metaphases (n) H^o	No. of abnormal metaphases	No. and % of metaphases with different types of chromosomal aberrations ^a					Mean % abnormal Metaphases $\pm$ S.E.
			X-Yu (%)	A-u (%)	X-Yu+A-u (%)	Fragment and/or break (%)	Chain IV (%)	
I. Control (non-treated)	500	18	11 (2.2)	7 (1.4)	—	—	—	3.60 ± 0.24
II. Potassium dichromate ($mg\ kg^{-1}\ b.wt.$)								
3	500	31	21 (4.2)	10 (2.0)	—	—	—	$6.20 \pm 0.89^*$
6	500	40	22 (4.4)	13 (2.6)	3 (0.6)	2 (0.4)	—	$8.00 \pm 0.45^{**}$
12	500	51	26 (5.2)	17 (3.4)	3 (0.6)	2 (0.4)	3 (0.6)	$10.20 \pm 0.58^{**}$
24	500	63	33 (6.6)	19 (3.8)	5 (1.0)	3 (0.6)	3 (0.6)	$12.60 \pm 0.45^{**}$
III. Thiola ($50\ mg\ kg^{-1}\ b.wt.$)	500	19	12 (2.4)	7 (1.4)	—	—	—	3.80 ± 0.37
IV. Potassium dichromate ($mg\ kg^{-1}\ b.wt.$)								
+ Thiola ($50\ mg\ kg^{-1}\ b.wt.$)								
6 + Thiola	500	22	18 (3.6)	3 (0.6)	—	—	1 (0.2)	4.40 ± 0.20
12 + Thiola	500	28	17 (3.4)	7 (1.4)	1 (0.2)	2 (0.4)	1 (0.2)	$5.60 \pm 0.40^*$
24 + Thiola	500	36	19 (3.8)	8 (1.6)	1 (0.2)	5 (1.0)	3 (0.6)	$7.20 \pm 0.37^{**}$
VII. Mitomycin C								
(Positive control) $1\ mg\ kg^{-1}\ b.wt.$	400	77	34 (8.5)	17 (4.3)	10 (2.5)	12 (3.0)	4 (1.0)	$19.25 \pm 0.98^{***}$

(n)=Number of animals per each treatment group=5, H^o =Harvest time after treatment=24 h, ^aX-Yu=X-Y univalent, A-u=Autosomal univalent. * Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level (t-test).

permanent fish cell line RTG-2 *in vitro* (Kohlpoth *et al.* 1999) and also in plant cells (Siegfried *et al.* 1998).

Changes in Ca^{2+} concentration can influence the induction of chromosomal aberrations induced by chromium compounds (Anghileri 1973), an effect probably related to the fact that calcium contributes to maintain the integrity of chromosome structure (Mazia 1954). Chromium has multiple effects in cells which may produce mutations in DNA (Casadevall *et al.* 1999). Chromium is known to increase the concentration of hydroxyl radicals in cells *via* the Fenton reaction path way, and these radicals may react with DNA causing both base damage and strand breaks (Aiyar *et al.* 1989, Strohs and Bagchi 1995). Additionally, it has been shown that exposure to chromium can lead to both DNA protein cross-linking (Mattagajasingh and Misra 1996) and DNA-DNA cross-linking (Xu *et al.* 1996). All of these effects of chromium treatment can lead to mutations in cellular genes and eventually results in cellular transformation.

Table 2 also shows that the potential of $\text{K}_2\text{Cr}_2\text{O}_7$ induced chromosomal aberrations was greatly reduced in groups treated i.p. with thiola $50 \text{ mg kg}^{-1} \text{ b.wt.}$ 2 h prior to the administration of the metal. Such decrease was found to be statistically significant compared with the respective groups treated with $\text{K}_2\text{Cr}_2\text{O}_7$ alone. A significant decrease of the structural chromosomal aberrations and a reduction of the incidence of C-mitosis have been obtained. The same protective effect of thiola was reported against the genotoxicity induced by benzo (a) pyrene in human embryonic lung fibroblasts of the cell line I CP-23 and in $\text{C}_{56\text{B1/6}}$ mouse bone-marrow (Galdean *et al.* 1986). Moreover a protective effect of thiola was also noted in human peripheral blood cells treated with different mutagens (Dallapiccola *et al.* 1985). The results illustrated in Tables 1 and 2 concerning the protective role of thiola indicate that the induction of chromosomal aberrations and SCE's by $\text{K}_2\text{Cr}_2\text{O}_7$ is partially based on different mechanisms.

The induction of chromosomal aberrations in mouse spermatocytes after single i.p. treatment with different doses of $\text{K}_2\text{Cr}_2\text{O}_7$ (3, 6, 12, $24 \text{ mg kg}^{-1} \text{ b.wt.}$) was also studied (Table 3). All the tested concentrations had induced a significant increase in the percentage of chromosomal aberrations in mouse spermatocytes with a dose-related relationship. Such percentage reached 6.20 ± 0.89 and 12.60 ± 0.45 after treatment with the lowest and the highest tested doses respectively compared with 3.60 ± 0.24 for the control. After treatment with different doses of $\text{K}_2\text{Cr}_2\text{O}_7$ chromosomal aberrations (Fig. 1g-i) were observed in mouse spermatocytes. Univalent formation was highly increased (X-Y and autosomal univalents). However, X-Y univalents were much more frequent. Breaks, fragments and translocations were also observed. The results also show that thiola significantly reduced the incidence of chromosomal abnormalities induced by $\text{K}_2\text{Cr}_2\text{O}_7$ in mouse spermatocytes. The mean percentage of aberrant metaphases reached 7.20 ± 0.37 after treatment with the highest tested dose of $\text{K}_2\text{Cr}_2\text{O}_7$ and $50 \text{ mg kg}^{-1} \text{ b.wt.}$ thiola compared with 12.60 ± 0.45 for those treated with the same dose of $\text{K}_2\text{Cr}_2\text{O}_7$ alone.

The above results suggest that the environmental contamination by potassium dichromate may bring about serious problems to human health including genetic hazards. The results also clearly illustrate the protective role of thiola which succeed for minimizing the incidence of chromosomal aberrations induced by $\text{K}_2\text{Cr}_2\text{O}_7$ in both somatic and germ cells of male mice.

References

- Aiyar, J., Borges, K. M., Floyd, R. A. and Wetterhahn, K. E. 1989. Role of chromium (V), glutathionethiol radical, and hydroxyl radical intermediates in chromium (VI)-induced DNA damage. *Toxicol. Environ. Chem.* **22**: 135-145.
- Alain, B., Occhipinti, P., Bartcai, E. and Orsiere, T. 1997. Genotoxic effects of potassium dichromate in cultured human lymphocytes. *Med. Sci. Res.* **24**: 797-799.
- Anghileri, L. J. 1973. Calcium metabolism in tumors. Its relationship with chromium complex accumulation. *Oncology* **27**: 30-44.
- Benjamin, S. and Stephen, J. 1996. Mitoclastic and clastogenic effects of aspirin on mouse bone-marrow cells *in vivo*.

- Cytologia **61**: 27–32.
- Bianchi, V., Celotti, L., Lanfranchi, G., Majone, F., Marin, G., Montaldi, A., Sponza, G., Tamino, G., Venier, P., Zantedeschi, A. and Levis, A. G. 1983. Genetic effects of chromium compounds. *Mut. Res.* **117**: 279–300.
- Biedermann, K. A. and Landolph, J. R. 1990. Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis, and anchorage independence in diploid human fibroblasts. *Cancer Res.* **50**: 7835–7842.
- Bonatti, S., Meini, M. and Abbondandolo, A. 1976. Genetic effects of potassium dichromate. *Mut. Res.* **38**: 147–150.
- Carlisle, D. L., Pritchard, D. E., Singh, J., Owens, B. M., Blankenship, L. J., Orenstein, J. M. and Patierno, S. R. 2000. Apoptosis and P53 induction in human lung fibroblasts exposed to chromium (VI): effect of ascorbate and tocopherol. *Toxicol. Sci.* **55**: 60–81.
- Casadevall, M., Fresco, P. and Kortenkamp, A. 1999. Chromium (VI). Mediated DNA damage: Oxidative pathways resulting in the formation of DNA breaks abasic sites. *Chemd. Biol. Interact.* **123**: 117–132.
- Dallapiccola, B., Porfrio, B., Mokini, V., Alimena, G., Isacchi, G. and Gandini, E. 1985. Effect of oxidants and antioxidants on chromosomal breakage in Fanconi anemia lymphocytes. *Hum. Genet.* **69**: 62–65.
- Evans, E. P., Breckon, G. and Ford, C. E. 1964. An air drying method for meiotic preparation from mammalian testes. *Cytogenetics* **3**: 289–294.
- Ezzat, I., El-Demerdash, S., Khorshid, A., Abu-Zeid, M., Aboul-Enein, M. and El-Merzabani, M. 1982. Radiation protection of thiola in mice: II-Effect on hematopoietic system and life-span after total body irradiation. *J. Egypt. Natl. Cancer Inst.* **1**: 77–85.
- Galdean, D., Petrasincu, D., Alangu, P., Ibric, S. and Voiculescu, N. 1986. The protective effect of thiola against the genotoxicity action of benzo (a) pyrene. *Experientia* **42**: 572–574.
- Goto, K., Maeda, S., Kano, Y. and Sugiyama, T. 1978. Factors involved in differential Giemsa staining of sister chromatids. *Chromosoma* **66**: 351–359.
- IARC. 1990. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 49. Chromium. Nickel and Welding. World Health Organization, Lyon. pp. 115–256.
- Itoh, S. and Shimada, H. 1998. Bone-marrow and liver mutagenesis in Laczz transgenic mice treated with hexavalent chromium. *Mut. Res.* **412**: 63–67.
- Knudsen, I. 1980. The mammalian spot test and its use for the testing of potential carcinogenicity of welding fume particles and hexavalent chromium. *Acta pharmacol. Toxicol.* **47**: 66–70.
- Kohlthop, M., Rusche, B. and Nusse, M. 1999. Flow cytometric measurement of micronuclei induced in a permanent fish cell line as a possible screening test for the genotoxicity of industrial waste waters. *Mutagenesis* **14**: 397–402.
- Leonard, A. and Lauwerys, R. R. 1980. Carcinogenicity and mutagenicity of chromium. *Mut. Res.* **76**: 227–239.
- Levis, A. G., Bianchi, V., Tamino, G. and Pegoraro, B. 1978a. Cytotoxic effects of hexavalent and trivalent chromium compounds on mammalian cells *in vitro*. *Br. J. Cancer* **37**: 386–396.
- , Buttignol, M., Bianchi, V. and Sponza, G. 1978b. Effect of potassium dichromate on nucleic acid and protein syntheses and on precursor uptake in BHK fibroblasts. *Cancer Res.* **38**: 110–116.
- Madrigal-Bujaidar, E., Diaz Barriga, S., Cassani, M., Molina, D. and Ponce, G. 1998. *In vivo* and *in vitro* induction of sister chromatid exchanges by nordihydroguaiaretic acid. *Mut. Res.* **412**: 139–144.
- Majone, F. 1977. Effects of potassium dichromate on mitosis of cultured mammalian cells. *Caryologia* **30**: 468–481.
- and Levis, A. G. 1979. Chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells treated *in vitro* with hexavalent chromium compounds. *Mut. Res.* **67**: 231–238.
- Mattagajasingh, S. and Misra, H. P. 1996. Mechanisms of the Chromium (VI)-induced DNA-protein cross-links and their characterization in cultured intact human cells. *J. Biol. Chem.* **271**: 33550–33560.
- Mazia, D. 1954: The particulate organization of chromosome. *Proc. Natl. Acad. Sci. (USA)* **40**: 521–527.
- Nakamuro, K., Yoshikawa, K., Sayato, Y. and Kurata, H. 1978. Comparative studies of chromosomal aberrations and mutagenicity of trivalent and hexavalent chromium. *Mut. Res.* **58**: 175–181.
- Newbold, R. F., Amos, J. and Connell, J. R. 1979. The cytotoxic, mutagenic and clastogenic effects of chromium containing compounds on mammalian cells in culture. *Mut. Res.* **67**: 55–63.
- Ohno, H., Hanaoka, F. and Yamada, M. 1982. Inducibility of sister chromatid exchanges by heavy metal ions. *Mut. Res.* **104**: 141–145.
- Russo, A., Dorigo, E., Stocco, A. and Majone, F. 1993. Persistence of chromosomal lesions induced in mouse bone-marrow cells by mitomycin C, as evaluated by SCE analysis. *Mut. Res.* **287**: 275–282.
- Schunck, C., Miura, K. F. and Obe, G. 1998. Thiopronin reduces the frequencies of neocarzinostatin induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells. *Mut. Res.* **412**: 207–212.
- Siegfried, K., Gottmann, E., Steinkellner, H., Fomin, A., Pickl, C., Paschke, A., Göd, R. and Kundi, M. 1998. Detection of genotoxic effects of heavy metal contaminated soils with plant bioassays. *Mut. Res.* **420**: 37–48.
- Singh, J., Pritchard, D. E., Carlisle, D. L., Mclean, J. A., Montaser, A., Orenstein, J. M. and Patierno, S. R. 1999. Internalization of carcinogenic lead chromate particles by cultured normal human lung epithelial cells: formation of intracellular lead. Inclusion bodies and induction of apoptosis. *Toxicol. Appl. Pharmacol.* **161**: 240–248.

- Strohs, S. J. and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* **18**: 321–336.
- Sugahara, T. and Srivastava, P. N. 1976. MPG (2-Mercaptopropionylglycine) A Review on Its Protective Action against Ionizing Radiations. International Atomic energy. Agency, Vienna. 77–87.
- Tsuda, H. and Kato, K. 1976. Potassium dichromate induced chromosome aberrations and its control with sodium in hamster embryonic cells *in vitro*. *Gann* **67**: 469.
- and — 1977. Chromosomal aberrations and morphological transformation in hamster embryonic cells treated with potassium dichromate *in vitro*. *Mut. Res.* **46**: 87–94.
- Uma Devi, P. and Prasanna, P. G. 1990. Radioprotective effect of combinations of WR-2721 and mercaptopropionylglycine on mouse bone-marrow chromosomes. *Rad. Res.* **124**: 165–170.
- Umeda, M. and Nishimura, M. 1979. Inducibility of chromosomal aberrations by metal compounds in cultured mammalian cells. *Mut. Res.* **67**: 221–229.
- Xu, J., Bubley, G. J., Detrick, B., Blankenship, L. J. and Patierno, S. R. 1996. Chromium (VI) treatment of normal human lung cells results in guanine-specific DNA polymerase arrest, DNA–DNA cross links, and S-phase blockage of cell cycle. *Carcinogenesis* **17**: 1511–1517.
- Yosida, T. H. and Amano, K. 1965. Autosomal polymorphism in laboratory bred and wild Norway rats, *Rattus norvegicus* found in Misima. *Chromosoma*. **16**: 658–667.
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