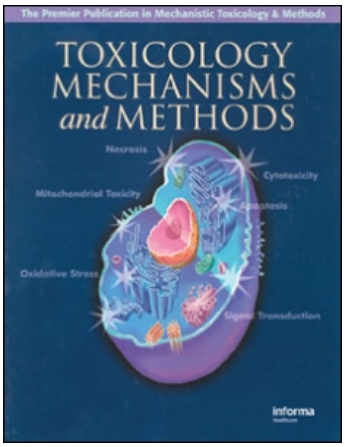

Toxicology Mechanisms and Methods
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713396575

Assessment of Cadmium Genotoxicity in Peripheral Blood and Bone Marrow Tissues of Male Wistar Rats

Ayla Çelik [a]; Belgin Büyükakilli [b]; Burak Çimen [c]; Bahar Tadelen [d]; M. kbal Öztürk [b]; Dilek Eke [a]
[a] Department of Biology, Faculty of Science and Letters, Mersin University, Mersin, Turkey [b] Department of Biophysics, Faculty of Medical Science, Mersin University, Mersin, Turkey [c] Department of Biochemistry, Faculty of Medical Science, Mersin University, Mersin, Turkey [d] Department of Biostatistic, Faculty of Medical Science, Mersin University, Mersin, Turkey

First Published:February2009

To cite this Article Çelik, Ayla, Büyükakilli, Belgin, Çimen, Burak, Tadelen, Bahar, Öztürk, M. kbal and Eke, Dilek(2009)'Assessment of Cadmium Genotoxicity in Peripheral Blood and Bone Marrow Tissues of Male Wistar Rats',Toxicology Mechanisms and Methods,19:2,135 — 140
To link to this Article: DOI: 10.1080/15376510802354979
URL: http://dx.doi.org/10.1080/15376510802354979

Toxicology Mechanisms and Methods, 19:135–140, 2009

ISSN: 1537-6516 print; 1537-6524 online
DOI: 10.1080/15376510802354979

informa
healthcare

Assessment of Cadmium Genotoxicity in Peripheral Blood and Bone Marrow Tissues of Male Wistar Rats

Ayla Çelik
Department of Biology, Faculty of Science and Letters, Mersin University, Mersin, Turkey

Belgin Büyükakilli
Department of Biophysics, Faculty of Medical Science, Mersin University, Mersin, Turkey

Burak Çimen
Department of Biochemistry, Faculty of Medical Science, Mersin University, Mersin, Turkey

Bahar Taşdelen
Department of Biostatistic, Faculty of Medical Science, Mersin University, Mersin, Turkey

M. İkbal Öztürk
Department of Biophysics, Faculty of Medical Science, Mersin University, Mersin, Turkey

Dilek Eke
Department of Biology, Faculty of Science and Letters, Mersin University, Mersin, Turkey

ABSTRACT In this study, Cadmium (Cd) genotoxicity was investigated in both bone marrow and peripheral blood treatment using rat micronucleus technique as genotoxicity test at acute and chronic treatment in the same animals. This study evaluated the frequency of micronuclei in the peripheral blood and bone marrow of male rats treated with unique cadmium dose (15 mg/kg. body w/day) by gavage for 60 days and acute treatment for 24 h, respectively. Mitomycin C (MMC) 2 mg/kg body wt was used as a positive control. This study shows that cadmium chloride treatment significantly induced the frequency of micronucleus in polychromatic erythrocytes in both tibia bone marrow and peripheral blood ($p < 0.001$, $p < 0.01$, respectively). This increase in micronucleus frequency shows that cadmium has a genotoxic effect on bone marrow and peripheral blood at this level. Also, in order to determine cytotoxicity in bone marrow and peripheral blood, the ratio of polychromatic erythrocytes to normochromatic erythrocytes was calculated in bone marrow and peripheral blood. Cd treatment decreased this ratio in only bone marrow. The results of this study demonstrate that Cd has both toxic and genotoxic potential in bone marrow and only genotoxic potential in peripheral blood. There is a significant difference between the control group and exposed group, including acute and chronic treatment for blood Cd level ($p < 0.001$). No significant difference was found between acute and chronic exposure group ($p > 0.05$).

KEYWORDS Cadmium; Genotoxicity; Micronucleus; Polychromatic Erythrocytes; Toxicity

INTRODUCTION

Cadmium (Cd) is a highly toxic heavy metal. Soluble compounds of cadmium (II) such as $CdCl_2$, are carcinogenic in experimental animals and humans (Oldiges et al. 1989; Stayner et al. 1992; Hartwig 1995). The genotoxicity and mutagenity, toxicity studies concerning Cd have produced controversial results depending on the assay used, in vivo and in vitro (Rozgaj et al. 1983; Han et al. 1992) . There are few studies related to the genotoxic activities in different tissues of Cd. Regarding the genotoxicity in bacterial and mammalian cell culture, the results are still conflicting; Cd (II) is not mutagenic in bacteria (Rossman et al. 1992), and the mutagenic effects are rather week in mammalian cells. In contrast, there are indications of rather indirect mechanisms of genotoxicity, perhaps due to an interaction with DNA repair processes (Hartwig 1994). There were reports of an increase in chromosomal aberrations in human peripheral blood lymphocytes after environmental (Tang et al. 1990) or occupational exposure to Cd. It is known that cadmium chloride induces aneuploidy in lymphocytes of exposed occupational people (Migliore and Nieri 1991) or in CHO cells (Seoane and Dulout 1994). Hurna and Hurna (2000) reported the

Received 2 May 2008;
accepted 21 July 2008.

Address correspondence to Ayla Çelik, PhD, Mersin University, Faculty of Science, Department of Biology, 33343 Mersin, Turkey. E-mail: mersin.edu.tr

increase in MN frequency in V79 cells induced by cadmium chloride. Otherwise, some recent publications did not confirm those findings (Van Hummelen and Kirsch-Volders 1992). Generally, the route and duration of exposure determine ultimately which organ becomes the target of Cd toxicity. Chronic exposure can lead to emphysema, obstructive airway disease, permanent renal dysfunction, or bone disorders immunosuppressant (Friberg et al. 1986). The results described so far demonstrate that cadmium (II) alone is genotoxic and enhances the genotoxicity of the other DNA damaging agents (Hartwig 1994). Hartwig and Beyersmann (1989) and Yamada et al. (1993) reported comutagenic and coclastogenic effects of cadmium in combination with chemical mutagens.

Usually only one in vivo test is required: typically a test for chromosome damage in the bone marrow of exposed rodents. Although a metaphase chromosome aberration test is sometimes considered, the micronucleus test is generally the preferred alternative method because it is less time-consuming, more sensitive, and can detect many aneuploidy-inducing agents.

The MN assay is the most widely used and best validated in vivo test for genotoxicity in dividing cells of various organisms in vivo and in vitro (Çelik et al. 2003, 2005a, 2005b; Dönmez-Altuntaş et al. 2006; Ünyayar et al. 2006). The in vivo MN assay has become increasingly accepted as the model of choice for evaluating the genotoxic potential of chemicals and/or radiation. The earliest applications of this model focused on the frequency of micronuclei in polychromatic erythrocytes (PCEs) of mouse bone marrow (Heedle 1973). Data were eventually developed showing that mouse peripheral blood is an acceptable cell population for detecting micronucleated PCEs (MNPCEs) as long as the sampling schedule accounted for the release of newly formed micronucleated erythrocytes from bone marrow to the blood (MacGregor et al. 1980; Schlegel and MacGregor 1982).

The purpose of the present work was to compare the genotoxic and cytotoxic effects of cadmium in the peripheral blood and bone marrow of laboratory rats using MN formation as the genetic endpoint in relation to exposure period.

MATERIALS AND METHOD

Chemicals

The standard solution of Cd was commercial preparations from Sigma Chemical Co. (St. Louis, Mo). $CdCl_2$ was dissolved in distilled water. Mitomycin C (MMC) (2 mg/kg b.w.) was used as positive control.

Animals and Treatment

The Local Ethics Committee for animal experiments in Medical Faculty of Mersin University approved this study. Procedures involving animals and their care conformed to institutional guidelines, in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research. In vivo studies were carried out on 12 male Wistar rats (Mersin University Medical Faculty, Mersin, Turkey), 3-months-old, at an initial body mass of 280–300 g. They were fed regular rat chow and water *ad libitum*. All the animals were kept in standard conditions with a 12:12 light:dark cycle, had unlimited access to standard diet and drinking water. The rats were allocated randomly to four experimental groups of four animals each: Positive control, non-treated rats; acute; and chronic groups. The chronic group was treated with cadmium chloride ($CdCl_2$) in 1 ml of distilled water (15 mg/kg body wt/day) for 60 days by oral gavage; The acute group was treated with a single dose of $CdCl_2$ in 1 ml of distilled water (15 mg/kg body wt) for acute exposures by oral gavage. It is acceptable that a positive control is administered by a route different from or the same as the test agent and that it is given only a single time (Hayashi et al. 1994). MMC was given as a single dose. The untreated control rats were treated identically with equal volumes of normal saline only via gavage throughout the study.

Tissue Preparation

All the animals used for experiments were anesthetized by ketamine hydrochloride (Ketalar, Eczacibasi Ilac Sanayi ve Ticaret A.S., Istanbul, Turkey). Blood samples were taken from their hearts into tubes. Then the tibiae were removed by dissection.

Micronucleus Assay

Whole blood smears were collected on the day following the last Cd administration or 1 day after MMC treatment. Whole blood smears were prepared on clean microscope slides, air-dried fixed in methanol, and stained with acridine orange (125 mg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope. The frequency of PCEs per total erythrocytes was determined using a sample size of 2000 erythrocytes per animal. The number of MNPCEs was determined using 2000 PCE per animal. Briefly, immature erythrocytes, i.e. PCEs, were identified by their orange–red color, mature erythrocytes by their green color, and micronuclei by their yellowish color.

The frequency of micronucleated erythrocytes in tibia bone marrow was evaluated according to the procedure of Schmid (1976), as performed in femoral bone marrow, with slight modifications. The bone marrow was flushed out from both tibias using 1 mL fetal bovine serum and centrifuged at 2000 rpm for 10 min. The supernatant was discarded. Bone marrow smears were prepared on clean microscope slides, air-dried, fixed in methanol, and stained with acridine orange (125 mg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluorescence microscope.

Scoring

In order to determine the frequency of micronucleus, 2000 PCEs per animals were scored to calculate the MN frequencies, and 200 erythrocytes (immature and mature cells) were examined to determine the ratio of PCE to normochromatic erythrocytes (NCEs) for bone marrow analysis. The frequency of PCEs per total erythrocytes was determined using a sample size of 2000 erythrocytes per animal. The number of MNPCEs was determined using 2000 PCE per animal for peripheral blood micronucleus test. Briefly, immature erythrocytes, i.e. PCEs, were identified by their orange–red color, mature erythrocytes by their green color, and micronuclei by their yellowish color.

TABLE 1 **Micronucleus induction and the PCE number induced by Cd treatment in bone marrow and peripheral blood of female Wista rat**

		Chronic treatment (peripheral blood)		Acute treatment (Bone marrow)	
Groups	Rat No	MN /2000 PCEs	PCE/2000 erythrocytes	MN /2000 PCEs	PCE/200 erythrocytes
Isotonic saline	1	2	2.8	2	108
	2	3	2.6	2	109
	3	3	2.85	3	104
	4	2	2.65	2	105
Mean ± SE		2.50 ± 0.57	2.7 ± 0.05	2.25 ± 0.25	106.5 ± 1.19
Cd (15 mg/kg bw)	1	4	2.25	4	76
	2	6	2	4	70
	3	6	1.9	5	79
	4	6	1.95	5	82
Mean ± SE		5.50 ± 0.50**	2.02 ± 0.07	4.25 ± 0.25***	76.75 ± 2.56***
MMC (2 mg/kg bw)	1	22	0.5	22	47
	2	20	0.6	24	45
	3	23	0.55	24	48
	4	25	0.5	23	46
Mean ± SE		22.5 ± 1.04***	0.53 ± 0.02***	23.25 ± 0.47***	46.5 ± 0.64***

MMC: Mitomycin C; MN: Micronucleus; PCE: Polychromatic erythrocyte; SE: Standard Error; bw: Body Weight.
**$p < 0.01$ and ***$p < 0.001$.

The Determination of Cadmium Concentration in Blood

Cd concentration of blood samples was determined by atomic absorption spectrometry (VARIANT GTA120 AA240-FS). The cathode lamp for Cd was operated under standard conditions using its resonance line of 228.8 nm. The Cd concentration was expressed in μg/L of blood.

Statistical Analysis

Data were compared by one-way variance analysis. Statistical analysis was performed using SPSS for Windows 9.05 package program. Multiple comparisons were performed by least significant difference test. $P < 0.05$ was considered to be the level of significance.

RESULTS

Table 1 represents micronucleus induction and the PCEs/NCEs ratios in bone marrow and peripheral blood. Cd treatment induced the frequency of MN in both rat bone marrow and peripheral blood. The mean of MN reached 5.50 ± 0.50 and 4.25 ± 0.25 in peripheral blood and rat bone marrow, respectively, while the frequency of MN was 2.5 ± 0.57, 2.25 ± 0.25 in peripheral blood and bone marrow control rats, respectively. Cd (15 mg/kg b.w) significantly induced the MN frequency compared to control in both rat bone marrow and peripheral blood ($p < 0.001$, $p < 0.01$, respectively) (Fig. 1).

In addition to this, the number of PCE measured in whole bone marrow and peripheral blood samples are summarized in Table 1. Cd treatment significantly decreased the PCE number when compared with controls in only bone marrow ($p < 0.001$). Cd is a toxic substance in both bone marrow at acute treatment and peripheral blood at chronic treatment. While PCE number was 2.5 ± 0.57 in the control group of chronic treatment, this value reached 5.5 ± 0.50 at chronic treatment. While PCE number was 106.5 ± 1.19 in the control group of acute treatment, this value reached 76.75 ± 2.56 at acute treatment.

Blood cadmium levels are presented in Table 2. In the control group, the level of Cd determined in blood Cd-exposed group was several times higher than in the control group (Fig. 2). There is a significant difference between control group and exposed group, including acute and chronic treatment, for blood Cd level ($p < 0.001$). No significant difference was found between the acute and chronic exposure group ($p > 0.05$).

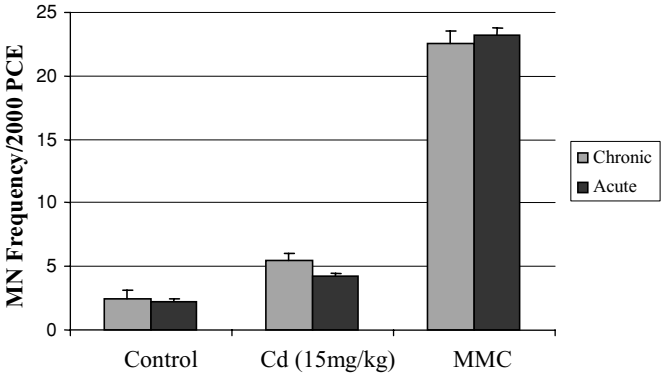

FIGURE 1 **MN frequency induced by Cd treatment.**

TABLE 2 **Blood cadmium levels from control and experimental groups**

Group	Rat number	Blood cadmium levels (μg/L)
Control	1	7.4
	2	10.1
	3	7.2
	4	8.1
Mean $\pm$ SD		8.2 $\pm$ 1.32
Acute Cd treatment	1	20.4
	2	15.6
	3	15.7
	4	15.9
Mean $\pm$ SD		16.9 $\pm$ 2.33***
Chronic Cd treatment	1	19.1
	2	18.3
	3	20.3
	4	22.1
Mean $\pm$ SD		19.9 $\pm$ 1.65***

***$p > 0.001$.

DISCUSSION

In the present study, the increase in micronucleus frequency revealed genotoxicity of Cd in both bone marrow and peripheral blood. Cd treatment significantly decreased the PCE number when compared with controls in only bone marrow ($p < 0.001$). Cd is a toxic substance in both bone marrow at acute treatment and peripheral blood at chronic treatment. An advantage of animal studies is that they provide a complete biological system which can evaluate the overall effect of subtle changes observed in cell systems. Carefully controlled animal studies are an essential step in the extrapolation of biological effects to human health safety. The fundamental similarities in cell structure and biochemistry between animals and humans provide a general valid basis for prediction of likely effects of chemicals on human populations. These data allow an estimate of the genetic risk of Cd exposure by using biomarkers such as MN. Genotoxicity activity is normally indicated by a statistically significant increase in the incidence of micronucleated immature erythrocytes for the treatment groups compared with the control group; historical vehicle/negative control results are also taken into account. Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of immature erythrocytes; a very large decrease in the proportion would be indicative of a cytostatic or cytotoxic effect. Pollution by heavy metals is an important problem due to their stable and persistent existence in the environment. Cadmium is one of the most toxic environmental and industrial pollutants affecting the DNA. Its ability to accumulate in living organisms is a significant threat to the environment and public health. The in vivo micronucleus test used in this study was a very sensitive method to evaluate the chromosomal damage in mammalian cells exposed to chemical substances. Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. Their presence in cells is a refection of structural and/or numerical chromosomal aberrations arising during mitosis (Heddle et al. 1991).

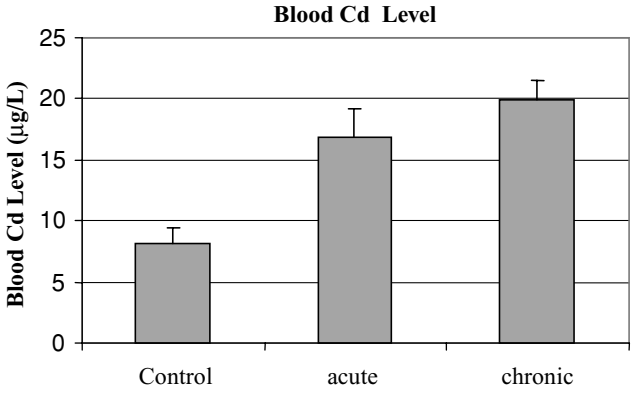

FIGURE 2 **Blood Cd levels.**

The studies performed using different types of mammalian cells have demonstrated that Cd may induce various genotoxic effects (Ochi et al. 1983; Mouron et al. 2001). Kasuba and Rozgaj (2002) showed cadmium chloride, regardless of concentration, applied in early S phase produced a significant increase in MN frequency in human peripheral blood lymphocytes. In plant studies; it is shown that cadmium and its salts may cause genetic damage in cells. Ünyayar et al. (2006) reported that cadmium nitrate induced the micronuclus formation in *Allium cepa* and *Vicia faba* root cells. Besides, high concentrations of Cd decreased the mitotic index and caused the delay in mitosis stages in both plants, mainly in V.faba. On the other hand, lipid peroxidation was significantly enhanced with external Cd in V.faba.

Yamada et al. (1993) showed that Cd^{2+} inhibits DNA prereplicational repair and perhaps DNA excision repair, thereby causing co-clastogenic effects. Inhibition of DNA repair has been recommended to occur when Cd^{2+} competes with Zn^{2+} for a common binding to enzymes involved in DNA synthesis. DNA damage induced by environmental mutagenic is repaired predominantly by nucleotide excision repair (NER). Diverse DNA repair systems have evolved to remove DNA lesions derived from exogenous and endogenous sources. With respect to endogenous DNA damage, DNA replication errors are corrected by the mismatch repair system and removed from the newly synthesized DNA strand shortly after DNA replication.

However, much is known about the ability of metallic compounds to induce genotoxic damage in bone marrow cells of laboratory animals. The data of this study indicated that cadmium chloride is a potent genotoxic agent in rat bone marrow and peripheral blood. Cadmium chloride significantly increased the frequency of micronuclei and chromosome aberration, particularly, single-stand break, in rat polychromatic erythrocytes of bone marrow. In the present study, the increase in DNA single-strand break indicates the interaction between DNA and $CdCl_2$. Although cadmium is suspected to have a spindle activity and is known for its likely carcinogenic, mutagenic, and teratogenic activity (IARC 1987, 1993a, 1993b), cytogenetic bioassay data are conflicting (Hartwig 1994; Verougstraete et al. 2002), probably because of the indirect effects of Cd. Indeed, even if the mechanisms underlying the toxic

effects of cadmium are still not well understood, Cd is known to induce DNA strand breaks and chromosomal aberrations, but its mutagenic potential is rather weak (Hartwig 1994; Waisberg et al. 2003).

Cadmium is also known to induce genotoxicity via oxidative stress, DNA binding, or inhibition of DNA repair activities (Hartwig 1998). It was suggested that the mechanism of cadmium genotoxicity is mainly conditioned by single strand breaks in DNA through the direct cadmium–DNA interactions as well as by the action of incision nucleases and/or DNA-glycosylase during DNA repair (Privezentsev et al. 1996).

The results of this present study show that the group exposed to Cd presents a significant 2-fold higher Cd concentration than controls. The measurement of exposure performed (Cd content in blood) does reflect the real exposure of rats. There is a parallel relationship between blood cadmium level and the increase in micronucleus frequency in both acute treatment and chronic treatment. In conclusion, in acute treatment and chronic treatment, the increase in micronucleus frequency and high blood Cd concentration shows that genotoxicity depends on Cd accumulation in blood. Our findings support the results of a study performed by Kasuba et al. (2002) and as understood from two studies, $CdCl_2$ is genotoxic by both acute and chronic treatment at the same dose (15 mg/kg). Further investigations of the influence of the cell cycle on the sensitivity to cadmium may be helpful to better understand genomic damage and reveal the relationship of oxidative stress and DNA damage.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Çelik, A., Mazmancı, B., Çamlıca, Y., Aşkın, A., and Çömelekoğlu, Ü. 2003. Cytogenetic effects of lambda-cyhalothrin on wistar rat bone marrow. *Mutat. Res.* 539:91–97.

Çelik, A., Mazmancı, B., Çamlıca, Y., Aşkın, A., and Çömelekoğlu, Ü. 2005a. Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar ratbone marrow by gavage administration. *Ecotoxicol. Environ. Safety* 61:128–133.

Çelik, A., Mazmancı, B., Çamlıca, Y., Aşkın, A., and Çömelekoğlu, Ü. 2005b. Induction of micronuclei by lambda-cyhalothrin on wistar rat bone marrow and gut epithelial cells. *Mutagenesis* 20:125–129.

Dönmez-Altuntas, H., Hamurcu, Z., Liman, N., Demirtas, H., and Imamoglu, N. 2006. Increased micronucleus frequency after oral administration of cadmium in dogs. *Biol. Trace Elem. Res.* 112:241–246.

Friberg, L., Elinder, C.-G., and Kjellstrom, T. 1986. In Friberg, L., Elinder, C.-G., Kjellstrom, T., and Nordberg, G. F. (Eds.), *Cadmium and health: a toxicological and epidemiological appraisal*, Volume II, CRC Press, Boca Raton, FL, pp. 1–246.

Han, C., Wu, G., Yin, Y., and Shen, M. 1992. Inhibition of germanium oxide of the mutagenicity of codmium chloride in various genotoxicity assay. *Food and Chem* Toxicol, 30: 521–524.

Hartwig, A. 1994. Role of DNA repair inhibition in lead and cadmium-induced genotoxicity: a review. *Environ. Health Perspect.* 102(Suppl 3):45–50.

Hartwig, A. 1995. Current aspects in metal genotoxicity. *BioMetals* 8:3–11.

Hartwig, A. 1998. Carcinogenicity of metals compounds: possible role of DNA repair inhibition. *Toxicol. Lett.* 102–103:235–239.

Hartwig, A., and Beyersmann, D. 1989. Comutagenicity and inhibition of DNA repair by metal ions in mammalian cells. *Biol. Trace Elem. Res.* 21:359–365.

Hayashi, M., Tice, R. R., Macgregor, J. T., Anderson, D., Blakey, D. H., Kirsch-Volders Jr., M., Oleson, F. B., Pacchierotti, F., Romagna, F., Shimada, H., Sutou, S., and Vannier, B. 1994. In vivo rodent erythrocyte micronucleus assay. *Mutat. Res.* 312:293–304.

Heedle, J. A. 1973. A rapid in vivo test for chromosomal damage. *Mutat. Res.* 18:187–190.

Heddle, J. A., Cimino, M. C., Hayashi, M., Romagna, F., Shelby, M. D., Tucker, J. D., Vanparys, P., and MacGregor, J. T. 1991. Micronuclei as an index of cytogenetic damage: past, present, and future. *Environ. Mol. Mutagen.* 18:277–291.

Hurna, E., and Hurna, S. 2000. Protective effect of zinc on Cadmium-induced micronuclei in V79 cells. *J. Trace Elem. Med. Biol.* 14:55–57.

IARC. 1987. *Monographs on the evaluation of carcinogenic risks to humans and their supplements*. Lists of Evaluations, IARC, Lyon. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, vols. 1–42, Suppl. No. 7. ISBN 92 832 1411 0, p. 440.

IARC. 1993a. *Monographs on the Evaluation of Carcinogenic Risks to Humans: Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry*, vol. 58, ISBN 92 832 1258 4, pp. 41–117.

IARC. 1993b. *Monographs on the Evaluation of Carcinogenic Risks to Humans: Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry*, vol. 58. ISBN 92 832 1258 4, pp. 119–237.

Kašuba, V., and Rozgaj, R. 2002. Micronucleus distribution in human peripheral blood lymphocytes treated in vitro with cadmium chloride in G0 and S phase of the cell cycle. *Chemosphere* 49:91–95.

Kasuba, V., Rozgaj, R., Sarıc, M. M., and Blanuša, M. 2002. Evaluation of genotoxic damage of cadmium chloride in peripheral blood of suckling Wistar rats. *J. Appl. Toxicol.* 22:271–277.

MacGregor, J. T., Wehr, C. M., and Gould, D. H. 1980. Clastogen-induced micronuclei in peripheral blood erythrocytes: the basis of an improved micronucleus test. *Environ. Mol. Mutagen.* 2:509–514.

Mavournin, H., Blakey, D. H., Cimino, M. C., Salamone, M. F., and Heddle, J. A. 1990. The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 239:29–80.

Migliore, L., and Nieri, M. 1991. Evaluation of twelve potential aneuptoidogenic chemicals by the in vitro human lymphocyte micronucleus assay. *Toxicol. In Vitro* 5:325–336.

Mouron, S. A., Golijow, C. D., and Dulout, F. N. 2001. DNA damage by cadmium and arsenic salts assessed by the single cell gel electrophoresis assay. *Mutat. Res.* 498:47–55.

Nocentini, S. 1987. Inhibition of DNA replication and repair by cadmium in mammalian cells. Protective interaction of zinc. *NucLeic Acid Res*. 15:4211–4225.

Ochi, T., Ishigura, T., and Oshawa, M. 1983. Participation of active oxygen species in the induction of single strand scissions by cadmium chloride in culture Chinese hamster cells. *Mutat. Res.* 122:169–175.

Oldiges, H., Hochrainer, D., and Glaser, U. 1989. Long-term inhalation study with Wistar rats and four cadmium compounds. *Toxicol. Environ. Mol. Mutagen.* 19:217–222.

Privezentsev, K. V., Sirota, N. P., and Gaziev, A. I. 1996. The genotoxic effects of cadmium studied in vivo. *Tsitol Genet.* 30:45–51.

Rossman, T. G., Roy, N. K., and Lin, W. 1992. Is cadmium genotoxic? In Nordberg, G. F., Alessio, I., Herber, R. F. M. (Eds.), *Cadmium 3. Trace ELem. Med. BioL. 16/3 (2002) in the human environment: Toxicity and Carcinogenicity*, International Agency for Research on cancer, Lyon, pp. 367–375

Rozgaj, R., Horvat, D., Racic, J., Bauman, A., and Pišl, Z. 1983. Cytogenetic effects of low dose ionizing radiation and heavy metals on cells in culture. *Period. Biol.* 85:367–375.

Schlegel, R., and MacGregor, J. T. 1982. The persistence of micronuclei in peripheral blood erythrocytes; detection of chronic chromosome breakage in mice. *Mutat. Res.* 104:367–369.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. In Hollaender A, editor. Chemical mutagen, principles and methods for their detection. Volume 4, New York: Plenum Press. 31–53.

Seoane, A. I., and Dulout, F. N. 1994. Use of the anaphase telophase test to detect aneugenic compounds: effects of propionaldehide and cadmiun chloride. *Bull. Environ. Contam. Toxicol.* 53:924–929.

Stayner, L., Smith, R. S., Thun, N., Schnorr, T., and Lemen, R. A. 1992. Dose-response analysis and quantitative assessment of Lung cancer risk and occupationaL cadmium exposure. *Ann. Epidem.* 2:177–194.

Tang, X. M., Chen, X. Q., Zhang, J. X. , and Qin, W. Q. 1990. Cytogenetic investigation in lymphocytes of people Living in cadmium polluted areas. *Mutat. Res.* 241:243–249.

Ünyayar, S., Çelik, A., Çekiç, F. Ö., and Güzel, A. 2006. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis* 21:77–81.

Van Hummelen, P., and Kirsch-Volders, M. 1992. Analysis of eight known or suspected aneugens by the in vitro human lymphocyte micronucleus test. *Mutagenesis* 7:447–455.

Verougstraete, V., Lison, D., and Hotz, P. 2002. A systematic review of cytogenetic studies conducted in human populations exposed to cadmium compounds. *Mutat. Res.* 1:15–43.

Waisberg, M., Joseph, P., Hale, B., and Beyersmann, D. 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology* 192:95–117.

Yamada, H., Miyahara, L., and Sasaki, Y. F. 1993. Inorganic cadmium increases the frequency of chemically induced chromosome aberrations in cultured mammalian cells. *Mutat. Res.* 302:107–145.