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Cytogenetic and microtubule array effects of the zineb-containing commercial fungicide formulation Azzurro® on meristematic root cells of *Allium cepa* L.

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ABSTRACT

Zineb [ethylene *bis*(dithiocarbamate) zinc] is a widely employed foliar fungicide for agricultural and industrial applications. *Allium cepa* L. is a reliable model for the assessment of xenobiotic genotoxicity and cytotoxicity. We evaluated the effects of the zineb-containing commercial formulation Azzurro® (70% zineb) in cell cycle stages of the meristem root cells of *A. cepa*. The mitotic index (MI), chromosomal aberrations at anaphase/telophase (CAs), micronuclei (MN), and abnormalities in immunodetected microtubule structures, e.g., preprophasic band (PPB), mitotic spindle (MS), and phragmoplast (Phrag), were used as end-points. Azzurro® (1 and 10 μg/ml) induced a significant increase in the frequency of CAs ($P<0.05$), and the higher concentration inhibited the MI ($P<0.05$) compared to control values. The frequency of MN did not differ from control values at any concentration. Treatment with 1 μg/ml Azzurro® induced a significant increase in the frequency of abnormal PPB ($P<0.01$), MS ($P<0.001$), and Phrag ($P<0.01$) and, at 10 μg/ml, enhancements in the frequencies of abnormal MS ($P<0.05$) and Phrag ($P<0.05$) were seen. A tubulin immunodetection assay showed that exposure to Azzurro® interferes with normal assembly of microtubule structures during mitosis.

1. Introduction

Carbamates are chemicals used in agriculture as insecticides, fungicides, herbicides, nematocides, or sprout inhibitors [1]. These chemicals are among the large group of synthetic pesticides that have been used on a large scale within the last 50 years. They are also used as biocides for industrial and household applications [1]. Large amounts of carbamate pesticides have been released into the environment. Humans may be exposed to them through food and drinking water around residences, schools, and commercial buildings [1]. Consequently, carbamates are potentially harmful to ecosystem health [2].

Among the carbamate pesticides, zineb [ethylene *bis*(dithiocarbamate) zinc] is a widely employed foliar fungicide with prime agricultural and industrial applications. Zineb has been registered for use on fruits, vegetables, field crops, ornamental plants, and for the treatment of many seeds. It has also been registered as a fungicide in paints and for mold control on fabrics, leather, linen, painted and wood surfaces, etc. [3].

Dithiocarbamates have given conflicting results in mutagenicity tests [1,4,5]. Available data on zineb do not allow a definitive evaluation of its carcinogenicity [1]. Zineb is considered non-mutagenic in bacterial systems, but this does not conclusively rule out damage to genetic material [1]. Possible genotoxic effects of zineb have been tested with regard to occupational exposure [6] and *in vitro* transforming properties [7]. In *Drosophila melanogaster*, zineb showed genotoxic effects in somatic and germ cells [8]. More recently, both *in vitro* monitoring of human peripheral blood lymphocytes and studies of CHO-K1 cells revealed its genotoxic effects by analyzing the frequency of biomarkers with inhibition of the mitotic activity, delay in cell cycle progression, and increase in the frequency of chromosomal aberrations and sister chromatid exchanges [9–11]. We have previously observed that immunodetection of β-tubulin allows characterization of mitotic spindle abnormalities induced in CHO-K1 cells by zineb and its Argentinean commercial formulation Azzurro® (zineb 70%) [11]. However, the mechanisms of chromosomal aberration induction are not known.

Immunodetection of tubulins has been used to characterize cytological changes induced by xenobiotics; effects are seen on architecture and cell function. Test systems have been developed, employing fungi and plant species, aimed at assessing the effects of

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agents on the cytoskeleton and mitotic apparatus. *Aspergillus nidulans*, *Allium cepa*, *Vicia faba*, *Hordeum vulgare*, and *Zea mays* are the most commonly used species [12–19]. In *A. nidulans*, effects on the dynamics of tubulin polymerization were studied following exposure to benzimidazoles and phenylalanine derivatives [15]. In *H. vulgare*, microtubule was evaluated following exposure to cadmium chloride, econazole nitrate, benomyl, thiabendazole, griseofulvin, thimerosal, and hydroquinone [14]. Another study determined the effects of cycloheximide on microtubule structures in *A. cepa* [12,13].

A. cepa L. is a suitable model for assessment of genotoxic compounds and complex mixtures [20–22] and for *in situ* analysis of contaminated soils [23–27]. This model is also widely used to investigate the role of microtubules on different structures involved within cell division [28–32]. These structures, known as preprophasic band (PPB), mitotic spindle (MS), and phragmoplast (Phrag) are organized sequentially during mitosis and participate in determining level of division, chromosome segregation, and cytokinesis, respectively.

We have evaluated the effects on mitotic index, chromosomal aberrations at anaphase/telophase, micronucleus, and immunodetected microtubule structures (PPB, MS, and Phrag) following exposure of *A. cepa* to the zineb-containing commercial formulation Azzurro® (70% zineb).

2. Materials and methods

2.1. Test chemicals

Azzurro® (70% zineb) was kindly provided by Chemiplant S.A. (Avellaneda, Buenos Aires, Argentina). Dimethyl sulfoxide (DMSO, CAS 67-68-5), bovine serum albumin (CAS 9048-46-8), mouse fluorescein isothiocyanate (FITC)-conjugated anti β-tubulin monoclonal antibody, propidium iodide (CAS 25535-16-4), 4′,6-diamidino-2-phenylindole (DAPI, CAS47165-04-8), and paraformaldehyde (CAS 68476-52-8) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Orcein (CAS 1400-62-0) was obtained from Sistemas Analíticos S.A. "Biopack" (Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina). Methanol (CAS 67-56-1), EGTA (CAS 67-42-5), cellulase (CAS 9012-54-8) and pectinase (CAS 9032-75-1) were obtained from Merck KGaA (Darmstadt, Germany), and PIPES (CAS 5625-37-6) was purchased from MP Biomedicals (Solon, OH, USA).

2.2. A. cepa meristematic cell source and fungicide treatment

Bulbs of onion (*A. cepa* L., $2n = 16$) were procured from a local market. Hand-picked bulbs of uniform size were scraped so that the apices of the root primordial were exposed and their dry scales peeled off. Bulbs were prepared by removing the cataphylls and rootlets of the growth plate, to avoid damage. After rinsing with tap water (1 h), three bulbs were placed in containers with filtered tap water for 24 h to stimulate rooting. Bulbs were then selected for root growth start and uniform growth length (1–2 mm). Roots were exposed for 30 h to Azzurro®, 1 or 10 μg/ml (23 °C, constant aeration and light protection). Azzurro® concentrations were selected according to previous studies on CHO-K1 cells [11]. Negative controls were processed concurrently.

2.3. Conventional cytogenetic analyses

Following fungicide treatment, roots were cut, fixed in ethanol:acetic acid (3:1), and stored in 70% ethanol (4 °C) until analyzed. Cytogenetic preparations of root meristem cells were performed using the conventional squash technique after digestion with ClH (1 N, 15 min) and staining with acetic orcein. Slides were coded and analyzed blind by one researcher with a 1000× optical light microscope (Leica DMLB). Parameters analyzed were mitotic index (MI), micronucleus (MN), and chromosomal aberrations at anaphase/telophase (CAs). The IM and MN genotoxicity values were determined by analyzing at least 1000 cells per onion for each experimental point; CA were determined by analyzing 100 mitoses per bulb. Chromosome abnormalities (acentric fragments, anaphases bridges, sticky chromosomes, C-metaphases, multipolar and disorganized anaphases, lagging chromosomes, chromosomal loss, and polyploid cells) were analyzed applying the criteria described by Fiskesjo [21]. The criteria employed for identifying MN were: diameter of MN varies between 1/16 and 1/3 of the diameter of the main nucleus; not refractive; not linked to the main nucleus. The MN may touch but not cover the nucleus and its membrane must be seen clearly. The MN staining is similar to the main nucleus but can sometimes be more intense [33]. MI were expressed as the number of dividing cells among 1000 scored cells. CAs were expressed as the total number of aberrations per 100 anaphases/telophases, as the percentage of total CAs. MN was expressed as the number of micronuclei per 1000 nuclei.

2.4. Slide preparation and direct immunofluorescence β-tubulin labeling

Following treatment, roots were fixed (45 min, room temp.) in 4% paraformaldehyde in microtubule stabilizing buffer (MSB) containing 50 mM PIPES, 5 mM $MgSO_4$ and 5 mM EGTA in distilled water, as recommended by Eleftheriou and Palevitz [32] with minor modifications. Fixation was followed by three washes with MSB (45 min each, room temp.). Afterwards, the MSB was discharged, slides were incubated with 10 μl MSB containing cellulase 2% P/V-pectinase 20% V/V (20 min, 37 °C) in a humidified atmosphere, and finally rinsed three times (5 min each) in MSB. The meristematic region was separated from the apex and placed flattened onto pre-cleaned slide. A coverslip was placed, the squash technique applied, and then the slides were fixed in methanol at −20 °C (15 min). After rinsing twice in MSB (15 min each), the cells were incubated with mouse FITC-conjugated anti-β-tubulin monoclonal antibody in a humidified atmosphere (37 °C, 3 h). The antibody was diluted 1:50 in MSB containing 3% bovine serum albumin. Subsequently, slides were rinsed three times with MSB (5 min each), air-dried at room temp., stained with either propidium iodide (0.1 μg/ml, 10 min) or DAPI (2.5 mg/ml diluted to 0.05% V/V), and mounted with an antifading medium (Vectaschield mounting medium H1000, Vector Laboratories, Burlingame, CA, USA). Slides were coded and scored blind by one researcher using a Leica DMLB fluorescence photomicroscope equipped with an appropriate filter combination and an integrated high-sensitivity monochrome charge-coupled device (CCD) camera (Leica DFC 340 FX).

2.5. Analysis of microtubule structures anomalies

Preprophasic bands (PPB), mitotic spindles (MS), and phragmoplasts (Phrag) were scored, according to recommendations reported elsewhere [14,34,35]. Incomplete or abnormal MS, tripolar spindles, incomplete PPB, and aberrant Phrag were considered as abnormal structures. A minimum of 1000 microtubule structures per sample were analyzed to determine the percentage of normal and abnormal figures and the type of anomalies. Data were expressed as the frequency of abnormal figures among 100 structures counted.

2.6. Statistical analysis

Results were analyzed using Student's *t*-test with Statistica software (Stat Soft 1999). The chosen level of significance was 0.05 unless indicated otherwise.

3. Results

3.1. Conventional cytogenetics

No differences in MI, MN and CAs were observed between untreated (negative control) and DMSO-treated cells (solvent control) ($P > 0.05$, Table 1). Both Azzurro® concentrations induced a significant increase in the frequency of CAs in anaphase–telophase ($P < 0.05$). Only the higher (10 μg/ml) concentration inhibited MI compared to control values ($P < 0.05$). The most frequently observed CAs consisted of abnormal anaphases and C-mitosis and, less often, rings, bridges and lagging chromosomes (Fig. 1). The frequency of MN did not differ from control values regardless of fungicide concentration ($P > 0.05$, Table 1).

3.2. Analysis of microtubule structures

No alterations among the frequencies of normal BPP (Figs. 2a and 3a-a′), MS (Figs. 2b and 3b-b′), and Phrag (Figs. 2c and 3c-c′) were observed between untreated (negative control) and DMSO-treated cells (solvent control) ($P > 0.05$, Table 2). Treatment with Azzurro®, 1 or 10 μg/ml, induced a significant increase in the frequency of abnormal PPB ($P < 0.01$), MS ($P < 0.001$), and Phrag ($P < 0.001$) (Table 2). Further analysis of the morphology of the microtubule structures showed that depolymerized PPB (Fig. 2d), depolymerized MS (Figs. 2e, 3d-d′, 3e-e′), and aberrant Phrag (Figs. 2f and 3f) were the most frequently observed abnormal structures after Azzurro® treatment, regardless of the concentration tested (Table 2).

Table 1
Mitotic index (MI), micronucleus (MN), anaphase–telophase aberrations (CA) in meristem root cells of *Allium cepa* exposed to Azzurro®.

Treatment	MI ± SD (%)	MN ± SD	Total CA					No of division cells	CA ± SD (%)
			Bridges	Ring	Lag	C-met	Dis An		
Control	7.50 ± 1.80	5.4 ± 4.7	-	-	-	4	8	955	1.32 ± 0.32
DMSO (1%)	7.53 ± 1.09	9.7 ± 10.0	-	-	-	3	4	870	1.17 ± 0.47
Azzurro (1 μg/ml)	6.50 ± 1.30	10.0 ± 4.2	8	7	17	25	54	900	12.85 ± 4.37*
Azzurro (10 μg/ml)	3.80 ± 0.20*	11.2 ± 4.2	8	8	9	25	27	913	8.46 ± 1.54*

Lag, lagging chromosome; C-met, C-metaphase; Dis An, disorganized anaphase. MI, expressed as percentage of mitoses among among 1000 interphase nuclei from each experimental point; MN, expressed as mean number of MN among 1000 interphase nuclei from each experimental point; CA expressed as number of aberrations among 300 anaphase–telophase per experimental point; SD, standar deviation; DMSO, solvent control.

* Student's *t*-test $p < 0.05$.

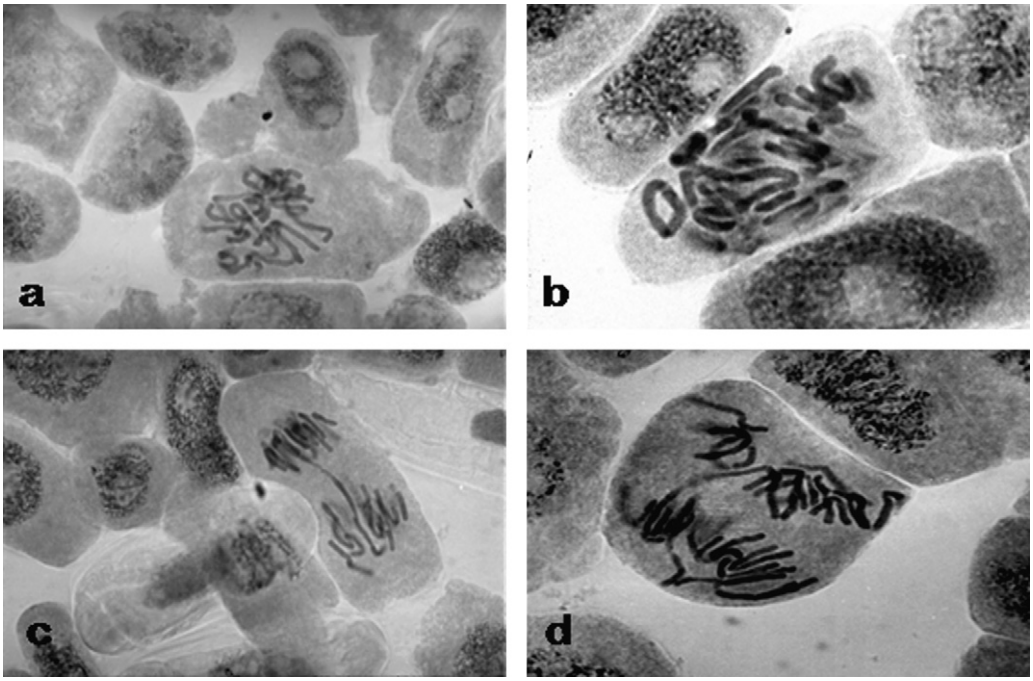

Fig. 1. *Allium cepa* meristematic cells exposed to Azzurro®. (a) Abnormal metaphase; (b) abnormal metaphase with ring chromosome and sticky chromosomes; (c) abnormal anaphase with bridge; (d) abnormal anaphase with vagrant chromosomes.

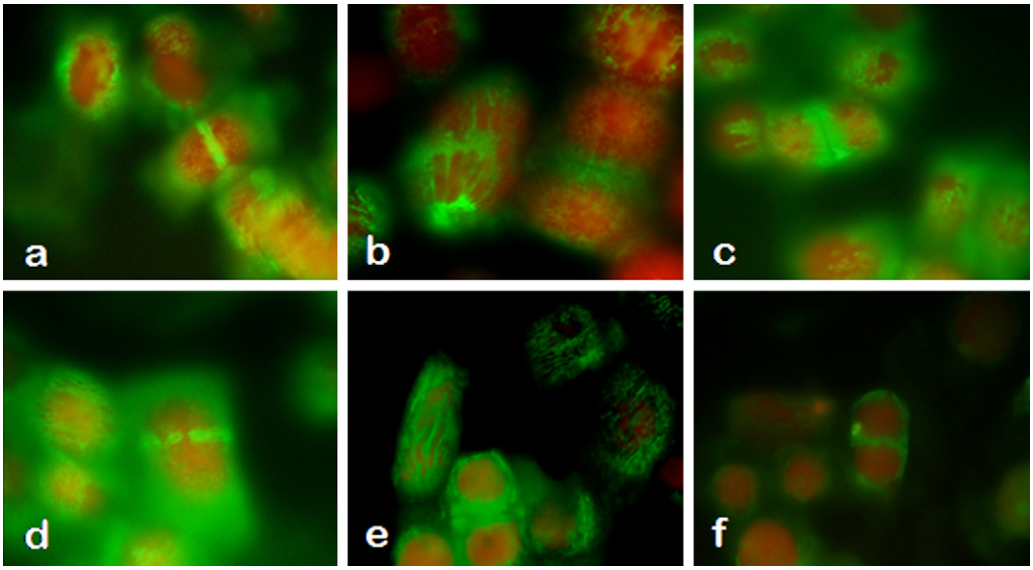

Fig. 2. Immunostaining of microtubule structures from *Allium cepa* meristematic cells with propidium iodide counterstaining. (a–c) Negative control cells; (a) preprophasic band; (b) mitotic spindle apparatus; (c) phragmoplast; (d and e) Cells exposed to Azzurro®; (d) depolimerized preprophasic band; (e) abnormal mitotic spindle; (f) abnormal phragmoplast (1000×).

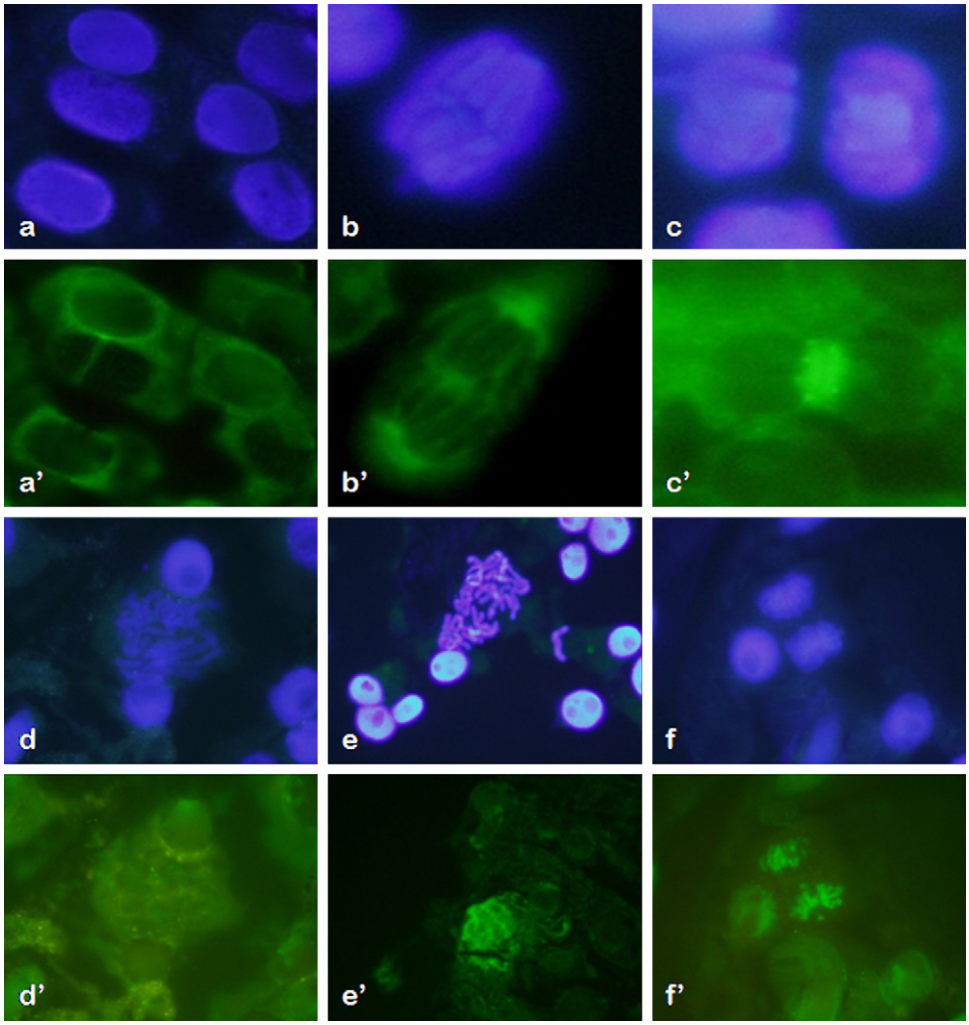

Fig. 3. Immunostaining of microtubule structures from *Allium cepa* meristematic cells. (a–c) Negative control cells stained with DAPI. (a′–c′) Negative control cells labeled with FITC conjugated anti-β-tubulin. (a) Prophase; (a′) preprophasic band; (b) anaphase; (b′) mitotic spindle; (c) telophase; (c′) phragmoplast; (d–f) Azzurro®-treated cells stained with DAPI; (d′–f′) Azzurro®-treated cells labeled with FITC conjugated anti-β-tubulin; (d) disorganized metaphase; (d′) depolimerized mitotic spindle; (e) disorganized anaphase; (e′) not formed mitotic spindle; (f) telophase; (f′) not formed phragmoplast (1000×).

Table 2
Frequency of abnormal microtubule arrays in meristematic root cells of *Allium cepa* exposed to Azzurro®.[a]

Treatment	PPB	MS	Phrag
Control	3.86 ± 3.41	–	3.03 ± 5.25
Azzurro 1 ug/ml	21.17 ± 5.52**	30.30 ± 3.60***	37.64 ± 7.26***
Azzurro 10 ug/ml	22.41 ± 2.51**	17.59 ± 6.99***	34.17 ± 8.04***

PPB, preprophasic bands; MS, mitotic spindle; Phrag, phragmoplas.
[a] Expressed as number of abnormal figures among 100 microtubule arrays analyzed.
** Student's-*t* test $p < 0.01$.
*** Student's-*t* test $p < 0.001$.

4. Discussion

Azzurro® induced cytotoxic and genotoxic damage in *A. cepa* meristematic root cells. The effect of the fungicide was evaluated using observation of abnormalities of the microtubule structures as endpoint. The method used provided a sensitive bioassay for detecting damage at the cellular level. These observations verify previous reports showing that microtubule structure anomalies are a reliable tool in genotoxicity and cytotoxicity studies in animal [11,36–43] or plant cells [44–47].

Azzurro® induced genotoxic effects, including disorganized anaphases, C-metaphases, chromosomal aberrations, and anaphase–telophase bridges, but not MN. Cytotoxic effects were observed as mitodepressive activity. These findings are consistent with observations previously reported by us in other *in vitro* systems, *i.e.*, human lymphocytes and hamster cells [9–11,48,49]. In cultured peripheral blood lymphocytes, the total number of chromosome- and chromatid-type aberrations was increased within the 0.1–100 μg/ml Azzurro® dose range, whereas a reduction of mitotic activity was observed with doses above 10 μg/ml [9]. Recently, we observed induction of MN when Azzurro®, 25 μg/ml, was added to cultures [49]. In CHO cells, equivalent concentrations significantly inhibited mitotic activity (1–25 μg/ml) and, in the comet assay, induction of DNA single-strand breaks was observed only at doses higher than 100 μg/ml [48].

The tubulin immunodetection assay showed that exposure to Azzurro® interferes with the formation of microtubule structures during mitosis. However, the results of the present study differ from those in CHO cells: multipolar spindles were present in CHO-treated cells but not in *A. cepa* [11]. Failure to induce multipolar spindles in *A. cepa* could be due to differences in the diversity of microtubule organizing centers in animal cells and vascular plants. Microtubule organizing centers of higher plants can be recognized by the presence of γ-tubulin [46,50]. Plant cells lack microtubule

organizing center comparable to centrosomes; instead, microtubule nucleation sites are distributed in the cytoplasm and in membranes such as the endoplasmic reticulum, nuclear envelope, or plastids. The microtubule organizing centers in seed plants are diffuse and migratory, which makes them difficult to identify, a major difference from centrosomes of animal cells [51].

Conflict of interest statement

The authors declare no conflict of interests.

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References

[1] IARC, Some carbamates, thiocarbamates and carbazides, International Agency for Research on Cancer, Lyon, 1976.
[2] EPA, ECOTOX: Ecotoxicology Database, USEPA/ORD/NHEERL, Mid-Continent Ecology Division, 2004, URL:http://www.epa.gov/ecotox/ecotox_home.htm.
[3] EPA, Compendium of Registered Pesticides, US Government Printing Office, Washington, DC, 1974.
[4] IARC, Occupational exposures in insecticide application and some pesticides, International Agency for Research on Cancer, Lyon, 1991.
[5] IARC, Genetic and related effects: an updating of selected IARC monographs from volumes 1 to 42, International Agency for Research on Cancer, Lyon, 1987.
[6] M.A. Pilinskaya, Results of cytogenetic examination of persons occupationally contacting with the fungicide zineb, Genetika 10 (1974) 140–146.
[7] P. Perocco, A. Colacci, B. Bonora, S. Grilli, In vitro transforming effect of the fungicides metalaxyl and zineb, Teratogen. Carcinogen. Mutagen. 15 (1995) 73–80.
[8] N.K. Tripathy, L. Dey, B. Majhi, C.C. Das, Genotoxicity of zineb detected through the somatic and germ-line mosaic assays and sex-linked recessive-lethal test in *Drosophila melanogaster*, Mutat. Res. 206 (1988) 25–31.
[9] S. Soloneski, M. González, E. Piaggio, M. Apezteguía, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. I. Genotoxic evaluation on cultured human lymphocytes exposed *in vitro*, Mutagenesis 16 (2001) 487–493.
[10] S. Soloneski, M. González, E. Piaggio, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. III. Genotoxic evaluation on Chinese hamster ovary (CHO) cells, Mutat. Res. 514 (2002) 201–212.
[11] S. Soloneski, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. V. Abnormalities induced in the spindle apparatus of transformed and non-transformed mammalian cell lines, Mutat. Res. 536 (2003) 121–129.
[12] Y. Mineyuki, H. Iida, Y. Anraku, Loss of microtubules in the interphase cells of onion (*Allium cepa* L.) root tips from the cell cortex and their appearance in the cytoplasm after treatment with cycloheximide, Plant Physiol. 104 (1994) 281–284.
[13] A. Nogami, T. Suzaki, Y. Shigenaka, Y. Nagahama, Y. Mineyuki, Effects of cycloheximide on preprophase bands and prophase spindles in onion (*Allium cepa* L.) root tip cells, Protoplasma 192 (1996) 109–121.
[14] G. Voutsinas, F.E. Zarani, A. Kappas, The effect of environmental aneuploidy-inducing agents on the microtubule architecture of mitotic meristematic root cells in *Hordeum vulgare*, Cell Biol. Int. 21 (1997) 411–418.
[15] T. Kiso, K.I. Fujita, X. Ping, T. Tanaka, M. Taniguchi, Screening for microtubule-disrupting antifungal agents by using a mitotic-arrest mutant of *Aspergillus nidulans* and novel action of phenylalanine derivatives accompanying tubulin loss, Antimicrob. Agents Chemother. 48 (2004) 1739–1748.
[16] W. Wang, R. Vignani, M. Scali, E. Sensi, M. Cresti, Post-translational modifications of α-tubulin in *Zea mays* L. are highly tissue specific, Planta 218 (2004) 460–465.
[17] M.I. Giménez-Abián, G. Giménez-Martín, M.H. Navarrete, C. De La Torre, Microtubular structures developed in response to a carbamate herbicide in plant mitosis, Protoplasma 200 (1997) 65–70.
[18] M. Fukuda, S. Hasezawa, N. Nakajima, N. Kondo, Changes in tubulin protein expression in guard cells of *Vicia faba* L. Accompanied with dynamic organization of microtubules during the diurnal cycle, Plant Cell Physiol. 41 (2000) 600–607.
[19] J.L. Evrard, I. Nguyen, M. Bergdoll, J. Mutterer, A. Steinmetz, A.M. Lambert, A novel pollen-specific α-tubulin in sunflower: structure and characterization, Plant Mol. Biol. 49 (2002) 611–620.
[20] H. Singh Rathore, M. Rathore, S. Panchal, M. Makwana, A. Sharma, S. Shrivastava, Can genotoxic effect be model dependent in *Allium* test? An evidence, Environ. Asia 3 (2010) 29–33.
[21] G. Fiskesjo, *Allium* test, in: S. O'Hare, C. Atterwill (Eds.), In Vitro Toxicity Testing Protocols, Springer, Heidelberg, Germany, 1995, pp. 119–127.
[22] J. Rank, L.C. Lopez, M.H. Nielsen, J. Moretton, Genotoxicity of maleic hydrazide, acridine, and DEHP in *Allium cepa* root cells performed by two different laboratories, Hereditas 136 (2002) 13–18.
[23] J.K. Katnoria, S. Arora, R. Bhardwaj, A. Nagpal, Evaluation of genotoxic potential of industrial waste contaminated soil extracts of Amritsar, India, J. Environ. Biol. 32 (2011) 363–367.
[24] O. Kovalchuk, P. Telyuk, L. Kovalchuk, I. Kovalchuk, V. Titov, Novel plant bioassays for monitoring the genotoxicity of drinking water from the inhabited areas of the Ukraine affected by the Chernobyl accident, Bull. Environ. Contam. Toxicol. 70 (2003) 847–853.
[25] O. Kovalchuk, I. Kovalchuk, A. Arkhipov, P. Telyuk, B. Hohn, L. Kovalchuk, The *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident, Mutat. Res. 415 (1998) 47–57.
[26] N. Andrioli, M. Mudry, Cytological and cytogenetic effects induced by thiabendazole on *Allium cepa* root meristems, Journal of basic applied genetics (BAG) in press.
[27] S. Cotelle, J.F. Masfaraud, J.F. Férard, Assessment of the genotoxicity of contaminated soil with the *Allium*/*Vicia*-micronucleus and the *Tradescantia*-micronucleus assays, Mutat. Res. 426 (1999).
[28] E.P. Eleftheriou, I.D.S. Adamakis, P. Melissa, Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of Allium cepa L, Protoplasma (2011), doi:10.1007/s00709-011-0292-3.
[29] M.I. Giménez-Abián, J.F. Giménez-Abián, L. Utrilla, C. De La Torre, Nuclear ploidy is contingent on the microtubular cycle responsible for plant cytokinesis, Protoplasma 224 (2004) 41–47.
[30] Y. Mineyuki, B.A. Palevitz, Relationship between preprophase band organization, F-actin and the division site in *Allium*. Fluorescence and morphometric studies on cytochalasin-treated cells, J. Cell Sci. 97 (1990) 283–295.
[31] J. Marc, B.A. Palevitz, Regulation of the spatial order of cortical microtubules in developing guard cells of *Allium*, Planta 182 (1990) 626–634.
[32] E.P. Eleftheriou, B.A. Palevitz, The effect of cytochalasin D on preprophase band organization in root tip cells of *Allium*, J. Cell Sci. 103 (1992) 989–998.
[33] M. Fenech, Cytokinesis-block micronucleus cytome assay, Nat. Protoc. 2 (2007) 1084–1104.
[34] Y. Mineyuki, S.M. Wick, B.E.S. Gunning, Preprophase bands of microtubules and the cell cycle: kinetics and experimental uncoupling of their formation from the nuclear cycle in onion root tip cells, Planta 174 (1988) 518–526.
[35] Y. Mineyuki, The preprophase band of microtubules: its function as a cytokinetic apparatus in higher plants, Int. Rev. Cytol. 187 (1999) 1–49.
[36] A.T. Natarajan, An overview of the results of testing of known or suspected aneugens using mammalian cells *in vitro*, Mutat. Res. 287 (1993) 113–118.
[37] U. Kochendörfer, I. Stammberger, D. Mayer, G. Schwanitz, A new possible parameter for the detection of aneuploidy inducing substances: the analysis of qualitative and quantitative abnormalities of the spindle apparatus, Mutat. Res. 361 (1996) 55–66.
[38] B.M. Miller, I.D. Adler, Suspect spindle poisons: analysis of c-mitotic effects in mouse bone marrow cells, Mutagenesis 4 (1989) 208–215.
[39] T. Ochi, Induction of multiple microtubule-organizing centers, multipolar spindles and multipolar division in cultured V79 cells exposed to diethylstibestrol, estradiol-17B and bisphenol A, Mutat. Res. 431 (1999) 105–121.
[40] T. Ochi, F. Nakajima, M. Nasui, Distribution of γ-tubulin in multipolar spindles and multinucleated cells induced by dimethylarsinic acid, a methylated derivative of inorganic arsenics, in Chinese hamster V79 cells, Toxicology 136 (1999) 79–88.
[41] T. Ochi, F. Nakajima, A. Shimizu, M. Harada, Induction of multinucleated cells in V70 Chinese hamster cells exposed to dimethylarsinic acid, a methylated derivative of inorganic arsenics: mechanisms associated with the formation of aberrant spindles, Toxicol. In Vitro 13 (1999) 11–25.
[42] T. Ochi, T. Suzuki, H. Isono, T. Kaise, In vitro cytotoxic and genotoxic effects of diphenylarsinic acid, a degradation product of chemical warfare agents, Toxicol. Appl. Pharmacol. 200 (2004) 64–72.
[43] E. Rojas, M. Valverde, L. Vega, A. Salvador, P. Ramirez, L.A. Herrera, D. Watters, M.F. Lavin, P. Ostrosky-Wegman, Genotoxic effects of bistratene A on human lymphocytes, Mutat. Res. 367 (1996) 169–175.
[44] Z. Vidaković-Cifrek, M. Pavlica, I. Regula, D. Papes, Cytogenetic damage in shallot (*Allium cepa*) root meristems induced by oil industry high-density brines, Arch. Environ. Contam. Toxicol. 43 (2002) 284–291.
[45] Y. Li, Y. Shen, C. Cai, C. Zhong, L. Zhu, M. Yuan, H. Ren, The type II *Arabidopsis* formin14 interacts with microtubules and microfilaments to regulate cell division, Plant Cell 22 (2010) 2710–2726.
[46] M. Pastuglia, J. Azimzadeh, M. Goussot, C. Camilleri, K. Belcram, J.-L. Evrard, A.-C. Schmit, P. Guerche, D. Bouchez, γ-Tubulin is essential for microtubule organization and development in *Arabidopsis*, Plant Cell 18 (2006) 1412–1425.
[47] M.I. Giménez-Abián, F. Panzera, J.F. López-Sáez, J.F. Giménez-Abián, C. De La Torre, G. Giménez-Martín, Immediate disruption of spindle poles and induction of additional microtubule-organizing centres by a phenylcarbamate, during plant mitosis, Protoplasma 204 (1998) 119–127.
[48] M. González, S. Soloneski, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. IV. DNA damage

and repair kinetic assessed by single cell gel electrophoresis (SCGE) assay on Chinese hamster ovary (CHO) cells, Mutat. Res. 534 (2003) 145–154.
[49] S. Soloneski, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. II. Clastogenesis on immunophenotyped human lymphocytes assessed by the micronucleus test, Environ. Mol. Mutagen. 40 (2002) 57–62.
[50] A.R. McDonald, B. Liu, H.C. Joshi, B.A. Palevitz, γ-Tubulin is associated with a cortical-microtubule-organizing zone in the developing guard cells of *Allium cepa* L, Planta 191 (1993) 357–361.
[51] B.A. Palevitz, Organization of the mitotic apparatus during generative cell division in *Nicotiana tabacum*, Protoplasma 174 (1993) 25–35.