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ScienceDirect
Toxicology in Vitro 20 (2006) 1481–1487
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Genotoxicity analysis of the phenoxy herbicide dicamba in mammalian cells in vitro

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Received 28 March 2006; accepted 3 May 2006
Available online 11 May 2006

Abstract

The cytogenetic effects exerted by the phenoxy herbicide dicamba and one of its commercial formulations banvel® (57.71% dicamba) were studied in in vitro whole blood human lymphocyte cultures. The genotoxicity of herbicides was measured by analysis of the frequency of sister chromatid exchanges (SCEs) and cell-cycle progression assays. Both dicamba and banvel® activities were tested within 10.0–500.0 μg/ml doses range. Only concentrations of 200.0 μg/ml of dicamba and 500.0 μg/ml of banvel® induced a significant increase in SCE frequency over control values. The highest dose of dicamba tested (500.0 μg/ml) resulted in cell culture cytotoxicity. The cell-cycle kinetics was affected by both test compounds since a significant delay in cell-cycle progression and a significant reduction of the proliferative rate index were observed after the treatment with 100.0 and 200.0 μg/ml of dicamba and 200.0 and 500.0 μg/ml of banvel®. For both chemicals, a progressive dose-related inhibition of the mitotic activity of cultures was observed. Moreover, only the mitotic activity statistically differed from control values when doses of both chemicals higher than 100.0 μg/ml were employed. On the basis of our results, the herbicide dicamba is a DNA damage agent and should be considered as a potentially hazardous compound to humans.

Keywords: Dicamba; Banvel; Chlorophenoxy herbicides; Sister chromatid exchanges; Cell-cycle kinetics

1. Introduction

It is well known that most pesticides in the environment have a toxic impact in non-target organisms, including man. Various reports have demonstrated all over the world that commonly used pesticides have non-desired genotoxic properties (IARC, 1991). Among these compounds, dicamba (3,6-dichloro-2-methoxybenzoic acid) is an herbicide widely utilized for the control of broadleaf weeds in wheat, corn, sugar cane, among other crops (IARC, 1991).

Dicamba has been profusely studied on its environmental aspects as seasonal leaching and biodegradation (Roy et al., 2001), degradation under anaerobic conditions (Gu et al., 2001), atmospheric transport and deposition (George et al., 2003) and remediation of dicamba-water contaminated (Gibb et al., 2004) but the available data on their molecular mechanisms of genotoxicity and mutagenicity are so far inconclusive (Perocco et al., 1990; Filkowski et al., 2003). Moreover, the unique ability of the plants to detect both double-strand breaks (homologous recombination) and point mutations provides tremendous potential in the study of molecular mechanisms of genotoxicity and mutagenicity of phenoxy herbicides (Filkowski et al., 2003).

Dicamba has been tested in different bioassays. In bacterial systems, it has proved to induce reverse mutations in several *Salmonella typhimurium* strains (Plewa et al., 1984) and DNA damage in *Bacillus subtilis* rec A and *Escherichia coli* pol A (Leifer et al., 1981; Waters et al., 1981). Several investigations using dicamba and/or the commercial formulation banvel® have been performed on plant assays such as *Arabidopsis thaliana* (Filkowski et al., 2003) and *Tradescantia* sp. (Mohammed and Ma, 1999). The most relevant

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doi:10.1016/j.tiv.2006.05.001

results from these investigations agree in demonstrating that this phenoxy herbicide has a significant effect on the frequency of homologous recombination A → G mutation and induces a dosage-related increase in the micronucleus frequencies, respectively.

Effect of the dicamba has also been evaluated in different animals systems. It produces peroxisome proliferation (Espandiari et al., 1995), loss of phosphorilation capacity in mitochondria (Peixoto et al., 2003) and has a promoting activity in two-stage hepatocarcinogenesis in rats (Espandiari et al., 1999). Specifically concerning DNA damage, dicamba failed to induce structural chromosome aberrations in rat bone marrow cells (Hrelia et al., 1994) or sex-linked recessive lethal mutations in *Drosophila melanogaster* (Waters et al., 1981) whereas it revealed its potential genotoxic hazard, assessed by diverse in vitro-tests (Perocco et al., 1990; Hrelia et al., 1994; Sorensen et al., 2005). In spite of the amount and variety of results on the genotoxicity of dicamba, they are not conclusive.

The aim of the present work was to assess the genotoxic effect of dicamba and its commercial formulation banvel® (dicamba 57.71%) on human lymphocytes cultures evaluating their genotoxic damage by the analysis of the sister chromatid exchange (James et al., 1997), cell-cycle progression (CCP) and mitotic index (MI) assays.

2. Materials and methods

2.1. Chemicals

Dicamba (3,6-dichloro-2-methoxybenzoic acid; CAS no. 1918-00-9) was obtained from Riedel-de-Haën (Pestanal®; Hannover, Germany). Banvel® (57.71% dicamba) was kindly provided by Syngenta Agro S.A. (Buenos Aires, Argentina). Dimethyl sulfoxide (DMSO) was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Blood samples

Blood samples were obtained from three healthy male non-smoking volunteer donors (age 20–30 years) selected according to previously described recommendations (Bianchi et al., 1979). Samples of 20 ml of blood were drawn from each donor by venipuncture immediately prior to culturing.

2.3. Whole blood lymphocyte cultures and herbicide treatment

Whole blood cultures were set up by seeding 1.0 ml of whole blood in 9.0 ml of complete culture medium [90% Ham's F10 (Gibco, Grand Island, NY), 10% fetal calf serum (Gibco), 100 U penicillin/ml (Gibco), 10 μg streptomycin/ml (Gibco)]. Immediately after seeding, dicamba, dissolved in DMSO, was diluted in culture medium so such addition of 100 μl to cultures would achieve the desired drug concentration. Banvel® (57.71% dicamba; excipients c.s.) was diluted in culture medium only. The final solvent concentration was <1% for all treatments. Dicamba and banvel® were used at final concentrations of 10.0, 20.0, 50.0, 100.0, 200.0 and 500.0 μg/ml. For each donor, two negative controls (untreated and solvent/vehicle-treated cultures, respectively) were performed and run simultaneously with dicamba- and banvel®-treated cultures from each donor. Cultures from each donor were established in duplicate for each treatment. None of the treatments produced significant pH changes in the culture medium, even at the highest concentration of dicamba or banvel®. Immediately after herbicide treatment, 0.3 ml of phytohaemagglutinin M (PHA M) (Gibco) and 10 μg BrdUrd/ml (Sigma Chemical Co.) were added to each culture (0 h). Afterwards, cultures were incubated at 37°C in a 5% CO_2 atmosphere for 72 h. During the last 3 h of culture cells were treated with 0.1 μg/ml colchicine (Sigma Chemical Co.). At the end of the culture period cells were harvested, exposed to a hypotonic solution (0.075 M KCl, 37 °C, 17 min) and fixed in methanol/acetic acid (3:1). Chromosome spreads were obtained using the air drying technique. The same batches of culture medium, sera and reagents were used throughout the study.

2.4. Fluorescence-plus-Giemsa (FPG) staining for sister chromatid differentiation

Chromosome spreads were stained using the FPG technique for sister chromatid differentiation as described in detail by Larramendy and Knuutila (1990). All cytogenetic slides were coded and scored blind by two cytogeneticists.

2.5. Cell-cycle kinetics and mitotic index

A minimum of 200 metaphase cells were scored per sample to determine the percentage of cells which had undergone one (M_1), two (M_2) or three or more mitoses (M_{3+}). All those metaphases showing differential staining of sister chromatids in <25% of the chromosomal complement were considered to be at the fourth cell cycle. The proliferative rate index (PRI) was calculated for each experimental point according to the formula $PRI = [(\%M_1) + 2(\%M_2) + 3(\%M_{3+})]/100$, which indicated the average number of times the cells had divided in the medium between the incorporation of BrdUrd and harvesting (Lamberti et al., 1983). The mitotic index (MI) was determined by scoring 2000 cells for each experimental point from each donor and was expressed as number of mitoses among 1000 nuclei. Changes in MI were expressed as a factor (f) of the mean MI of treated cultures (MI_t) over the mean MI form controls (MI_c) ($f = MI_t/MI_c$) (Miller and Adler, 1989).

2.6. Sister chromatid exchange analysis

For the SCE assay a total of 50 well-spread diploid metaphases were scored per treatment for each donor in M_2 cells. The data were expressed as the mean number of SCEs per cell ± SE.

2.7. Statistical analysis

The Kuskal–Wallis one way analysis of variance was used to compare differences among donors and treatments. The two-tailed Student's *t*-test was used to compare SCE frequencies between treated and control groups. A χ^2 test was used for cell cycle progression and MI data. The level of significance chosen was 0.05 unless indicated otherwise.

3. Results

Table 1 shows the frequencies of SCEs obtained in whole blood cultures for a 72 h continuous dicamba and banvel® treatments. Since no differences in the SCEs were observed between untreated and DMSO-treated cultures (negative controls), pooled data are presented for control cultures.

For dicamba, only the 200.0 μg/ml dose induced a significant increase in the SCEs frequency over control values (donor 1, $P \leqslant 0.01$; donors 2 and 3, $P \leqslant 0.05$). When a 500.0 μg/ml dose was used, a cytotoxic effect was observed, not allowing the SCEs frequency evaluation. Instead, banvel® induced a significant SCEs frequency increase over control values only when 500.0 μg/ml were employed ($P \leqslant 0.001$). However, for one of the donors (donor 2), the same dose of the test compound resulted in cellular death and thus the SCEs frequency could not be determined.

Both test compounds induced cytotoxicity in lymphocyte cultures since either a significant delay in cell-cycle progression or a significant reduction of the PRI were observed in those dicamba- and banvel®-treated cultures (Tables 2 and 3, respectively). As shown in Table 2, the 100.0 μg/ml dicamba concentration caused a significant increase of M_1 frequency (donor 1, $P \leqslant 0.001$; donors 2 and 3, $P \leqslant 0.05$) in relation to a solely significant M_{3+} decrease (donor 1, $P \leqslant 0.01$; donor 2, $P \leqslant 0.001$; donor 3, $P \leqslant 0.05$). When 200.0 μg/ml of dicamba were used, the cell-cycle alteration for donors 1 and 2 was due to significant increases of both M_1 and M_2 (donor 1, M_1 and M_2: $P \leqslant 0.001$, donor 2, M_1: $P \leqslant 0.001$, M_2: $P \leqslant 0.01$) in relation to a decrease in M_{3+} (donors 1 and 2, $P \leqslant 0.001$). At the same dose, donor 3 also showed a significant increase in M_1 ($P \leqslant 0.001$) due to a M_2 significant decrease ($P \leqslant 0.01$). Furthermore, for the highest dicamba dose tested (500.0

Table 1
Sister chromatid exchange (SCE) frequencies in control and dicamba- and banvel®-treated human lymphocytes[a]

Dose (μg/ml)	SCE frequencies[b]					
	Dicamba			Banvel® (57.71%)		
	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
0	7.44 ± 0.52	8.08 ± 0.56	7.28 ± 0.46	7.00 ± 0.59	6.32 ± 0.51	7.60 ± 0.53
10	8.56 ± 0.65	6.72 ± 0.39	7.56 ± 0.51	8.32 ± 0.50	7.24 ± 0.41	7.72 ± 0.43
50	7.04 ± 0.41	6.74 ± 0.70	7.48 ± 0.64	7.44 ± 0.51	6.36 ± 0.50	6.32 ± 0.35
100	7.64 ± 0.52	6.92 ± 0.49	8.20 ± 0.79	8.24 ± 0.45	6.92 ± 0.33	6.36 ± 0.56
200	10.32 ± 0.71**	9.44 ± 0.40*	8.78 ± 0.57*	7.80 ± 0.70	6.40 ± 0.50	8.52 ± 0.60
500	No cell division	No cell division	No cell division	11.95 ± 0.86***	ND	10.64 ± 0.47***

ND, not determined.
[a] Lymphocytes were treated with dicamba and banvel® immediately after stimulation with PHA and harvested 72 h later.
[b] Results are expressed as mean SCE/cell ± SE.
* $P \leqslant 0.05$.
** $P \leqslant 0.01$.
*** $P \leqslant 0.001$.

Table 2
Cell-cycle progression and proliferative rate index (PRI) values in control and dicamba-treated human lymphocytes in vitro

Dose (μg/ml)	Cell-cycle progression[a]											
	Donor 1				Donor 2				Donor 3			
	M_1	M_2	M_{3+}	PRI	M_1	M_2	M_{3+}	PRI	M_1	M_2	M_{3+}	PRI
0	12	32	56	2.44	10	38	52	2.42	18	41	41	2.23
10	13	42	45	2.32	12	32	56	2.44	18	29	53	2.35
50	17	27	56	2.39	8	38	44	2.16	19	33	48	2.29
100	29***	37	34**	2.05*	33*	31	36***	2. 03*	27*	47	26*	1.99
200	39***	51***	10***	1.71***	38***	56**	6***	1.68***	46***	22**	32	1.86*
500	No cell division			ND	No cell division			ND	No cell division			ND

ND, not determined.
[a] The proportion of cells in first (M_1), second (M_2), and third or subsequent cell divisions (M_{3+}) were determined in 300 mitoses for each experimental point.
* $P \leqslant 0.05$.
** $P \leqslant 0.01$.
*** $P \leqslant 0.001$.

Table 3
Cell-cycle progression and proliferative rate index (PRI) values in control and banvel®-treated human lymphocytes in vitro

Dose (μg/ml)	Cell-cycle progression[a]											
	Donor 1				Donor 2				Donor 3			
	M_1	M_2	M_{3+}	PRI	M_1	M_2	M_{3+}	PRI	M_1	M_2	M_{3+}	PRI
0	21	39	40	2.19	16	46	38	2.22	18	42	40	2.22
10	19	47	34	2.15	10	44	46	2.36	17	31	52	2.35
50	15	37	48	2.33	12	58	30	2.18	16	46	38	2.22
100	13	40	47	2.34	14	40	46	2.38	19	41	40	2.21
200	30*	42	28*	1.98	34***	30*	36	2.02	19	55*	26*	2.07
500	76***	24*	0***	1. 24***	98***	2***	0***	1.02***	58***	36	6***	1.48***

ND, not determined.
[a] The proportion of cells in first (M_1), second (M_2), and third or subsequent cell divisions (M_{3+}) were determined in 300 mitoses for each experimental point.
* $P \leqslant 0.05$.
** $P \leqslant 0.01$.
*** $P \leqslant 0.001$.

Table 4
Mitotic indices (MI) and proliferative factors (*f*) in control, dicamba- and banvel®-treated human lymphocytes[a]

Dose (μg/ml)	Cell-cycle progression											
	Dicamba						Banvel® (57.71%)					
	Donor 1		Donor 2		Donor 3		Donor 1		Donor 2		Donor 3	
	MI	*f*	MI	*f*	MI	*f*	MI	*f*	MI	*f*	MI	*f*
0	93	1.00	71	1.00	120	1.00	110	1.00	80	1.00	112	1.00
10	94	1.01	78	1.10	137	1.10	90	0.82	97	1.21	105	0.94
50	104	1.12	58	0.82	140	0.82	127	1.15	97	1.21	120	1.07
100	71*	0.76	44**	0.62	122	0.62	89*	0.81	57*	0.71	102	0.91
200	19***	0.20	16***	0.23	74***	0.23	43***	0.39	55**	0.69	85*	0.76
500	ND	ND	ND	ND	ND	ND	16***	0.15	2***	0.03	62***	0.55

ND, not determined.
[a] Lymphocytes were treated with dicamba and banvel® immediately after stimulation with PHA and harvested 72 h later.
* $P \leqslant 0.05$.
** $P \leqslant 0.01$.
*** $P \leqslant 0.001$.

μg/ml), cytotoxicity was observed since a complete cellular death was found. In regard to the PRI analysis, a significant reduction was observed with respect to control values for 100.0 (donors 1 and 2) and 200.0 μg/ml (donors 1–3) of dicamba doses (Table 2, donors 1 and 2: $P \leqslant 0.05$ and $P \leqslant 0.001$; donor 3: $P > 0.05$ and $P \leqslant 0.05$). Treatment with commercial banvel® yielded similar results (Table 3). At 200.0 μg/ml dose a significant increase in M_1 (donor 1, $P \leqslant 0.05$; donor 2, $P \leqslant 0.001$) concomitantly with a significant decrease of M_{3+} and M_2 for donor 1 and 2, respectively were found ($P \leqslant 0.05$). In donor 3, the M_2 increase was related only to a significant decrease in the proportion of M_{3+} ($P \leqslant 0.05$). Conversely to the effect found for the highest dose of the pure compound, 500.0 μg/ml of banvel® did not result in cell death. For this concentration a significant M_1 increase associated to a significant decrease in the frequencies of M_2 and M_{3+}, was observed for all donors (donor 1, $P \leqslant 0.05$ and 0.001; donors 2 and 3, $P \leqslant 0.001$). Likewise, the 500.0 μg/ml banvel® concentration induced a significant diminution of the PRI over control values (donor 3, $P \leqslant 0.001$).

The MI and *f* data for both dicamba- and banvel®-treated cultures are presented in Table 4. For dicamba, a dose-dependent inhibition of mitotic activity was observed when 100.0 and 200.0 μg/ml were used. The 100.0 μg/ml pure compound concentration produced a significant decrease of lymphocytes mitotic activity over control values only for donors 1 and 2 ($f = 1.00$) by a mean *f* of 0.69. For donor 3 no significant differences were found at 100 μg/ml of dicamba ($P > 0.05$). Likewise, the 200.0 μg/ml pure compound dose caused a significant reduction in the lymphocytes proliferation ($P \leqslant 0.001$) over control values for all donors ($f = 1.00$) by a mean *f* of 0.22 (range 0.20–0.23).

For the commercial formulation, the mitotic activity in treated lymphocytes showed an overall decrease in the 100.0–500.0 μg/ml dose range. The 100.0 μg/ml technical formulation concentration produced a significant mitotic activity reduction of cultures over control values solely for donors 1 and 2 ($f = 1.00$) by a mean *f* of 0.76. For donor 3 no significant differences were found at 100 μg/ml of banvel® ($P > 0.05$). The 200.0 μg/ml banvel® concentration resulted in a significant mitotic activity reduction over

control values ($f = 1.00$) by a mean f of 0.61 [$f = 0.39$ ($P \leqslant 0.001$), $f = 0.69$ ($P \leqslant 0.01$), and $f = 0.76$ ($P \leqslant 0.05$), for donors 1, 2, and 3, respectively]. Similar results were observed for the 500.0 μg/ml banvel® dose over control values ($f = 1.00$); when this concentration was employed, the lymphocyte mitotic activity decreased significantly ($P \leqslant 0.001$) by a mean f of 0.24 (range 0.03–0.55) over control values ($f = 1.00$).

4. Discussion

In the present report, the genotoxicities of herbicide dicamba and the dicamba containing commercial formulation banvel® were evaluated in in vitro cultures of human lymphocytes using different genetic end-points, namely, the frequency of SCEs in second mitosis, mitotic index, and the kinetics of cell cycle progression. The results demonstrate that dicamba at a 200.0 μg/ml concentration induced a significant increase in the frequency of SCEs whereas the highest dose tested (500.0 μg/ml) resulted in cell culture cytotoxicity. On the other hand, the commercial formulation banvel® was observed to display genotoxic effects by a significant enhancement of SCEs frequency only for the 500 μg/ml dose. When not cytotoxic, both chemicals altered the cell cycle progression in the 100.0–500.0 μg/ml dose range.

Reports on the in vitro effect on dicamba are scarce and have focused mainly on the genotoxicity aspects assessed by unscheduled DNA synthesis, sister-chromatid exchanges in human peripheral blood lymphocytes, structural chromosome aberrations in rat bone marrow (Hrelia et al., 1994) and more recently by single cell gel electrophoresis assay on Chinese hamster ovary (CHO) cells (Sorensen et al., 2005). As an alternative approach we used the kinetics of cell cycle-progression as another end-point for the first time in order to quantify genotoxicity. Doses of 100.0 and 200.0 μg/ml of dicamba resulted in the alteration of the cell-cycle progression or a significant decrease of the PRI. The PRI values changes were related to a varied combinations of first, second or third reduction and/or increases for the different donors. Similarly, for the same pure compound doses the MI and f factor revealed a valuable reduction. Such results remind that lymphocyte proliferation is an extremely complex system influenced by many factors as previously reported (Bianchi et al., 1979; Holland et al., 2002) and, at the same time, suggest that some individuals might be more sensitive to dicamba exposure than others. Particularly, we have previously assumed that different culture conditions including type of culture medium and sera, different response to the stimulation with PHA could be the cause of the inter-individual variability observed (Bianchi et al., 1979). However, this explanation can be ruled out for since the same batches of culture medium, sera and reagents were used throughout our study. Recently, Norppa (2004) proposed that the presence of genetic polymorphisms in same genes, e.g., *GSTM1*, *GSTT1*, *CYP1A1*, *NAT2* can affect cytogenetic biomarkers such as chromosomal aberrations. Furthermore, for some chemicals, it has been documented a genotype-dependent response to in vitro lymphocytes chemical treatment (Norppa et al., 1995; Wiencke et al., 1991). This plausible explanation could also be responsible for the variability observed in the mitotic indices found among the donors we included in the present study. Following the observations of Norppa (2004), it could be suggested that gene/genes involved in the in vitro activation of dicamba could also being affected by genetic polymorphism/s responsible for the differential response of different donors to dicamba. So far, no gene/s have been found to be responsible for the activation of dicamba. Whether some of the aforementioned genes were polymorphic in all or some of the donors we studied is still an open question.

Previous investigations on dicamba reveal its potential genotoxic effect on human lymphocytes. Perocco et al. (1990) and Hrelia et al. (1994), evaluated the genotoxic properties of dicamba in human whole blood and purified leukocyte cultures with and without the presence of the S-9 fraction. According to their findings, it has been suggested that dicamba does not require metabolic activation to exert its deleterious effects of lymphocytes in culture. Consequently, our experiments were carried out without the presence of the S-9 fraction during the culture period. Thus, it can be assumed that the genotoxic effect reported in the aforementioned studies as well as our results is exerted most probably by dicamba itself and not by any metabolites or any other subproducts of the compound generated during metabolic activation.

The scarce in vitro genotoxic knowledge on the dicamba properties extends to its technical formulation banvel®. Only its highest dose employed induced an increase of the SCEs frequencies whereas PRI, MI and f factor also showed a reduction. Although it is not available to us which are the excipients involved in the commercial formulation rather than 57.71% dicamba, our results clearly showed that any of them possesses any genotoxic effects, at least with the bioassays employed in the present study. Finally, our results demonstrated that both tested compounds were able to exert cytotoxicity effect, at least with doses higher than 100 μg/ml in the culture conditions we employed.

In our experiments both the effects of dicamba and banvel®, were assessed in whole blood lymphocyte cultures to exploit the metabolic capability of whole blood compared to other erythrocyte-free system like purified lymphocyte cultures (Norppa et al., 1980; Abdel-Fadil et al., 1982; Soloneski et al., 2001, 2002). Previous reports have suggested a protective role for the erythrocytes contained in whole blood cultures against the induction of genotoxic damage (Ray and Altenburg, 1978; Norppa et al., 1983; Larramendy and Reigosa, 1986; Soloneski et al., 2001, 2002). All these authors agree in demonstrating that the protective effect exerted by red blood cells, when present in culture media, is being most probably due to the antioxidant enzymes contained in these cells (Ray and Altenburg, 1978; Altenburg et al., 1979). From these observations, taking

into account the type of culture we employed in our experiments, it could be hypothesized that the genotoxic effect induced by dicamba, at least by using the end-points we have employed, could be accounted for active oxygen species delivered in vitro. However, further experiments are required to be performed in order to prove or discard this plausible explanation.

Chlorophenoxylic compounds like 2,4-dichlorophenoxyacetic acid (2,4-D) have been proved to cause lipid peroxidation in bacterial cellular envelope (Balague et al., 2001) and in human erythrocyte membranes (Duchnowicz and Koter, 2003). Dicamba also belongs to the chlorophenoxylic herbicide group and as far as it is known, the pesticide induces tissue damage and cell death in cleavers (*Galium aparine* L.) by lipid peroxidation (Grossmann et al., 2001). These facts lead to a strong suggestion for the dicamba damage mechanism by lipid peroxidation.

Dicamba has not been referenced as a carcinogen according to available sources and actually it is placed into group 3—not classifiable as to its carcinogenicity in humans—(EPA, 1974). The present study provides additional information on dicamba and banvel® genotoxicity and further analyses are required in order to achieve a better knowledge of the possible mechanism/s through these phenoxy compounds exert their genotoxic effects and to evaluate their mutagenicity/carcinogenicity in mammalian cells.

Acknowledgements

We thank M. Reigosa Ph.D. and M. Isla Larrain Ph.D., for technical assistance. This study was supported by Grants from the National University of La Plata (Grant number 11/N325), National Agency of Scientific and Technological Promotion (Contract Grant number PICT 2004 no. 26116) and National Council of Scientific and Technological Research (CONICET) from Argentina.

References

Abdel-Fadil, M.R., Palmer, C.G., Heerena, N., 1982. Effect of temperature variation on sister-chromatid exchange and cell-cycle duration in cultured lymphocytes. Mutation Research 104, 267–273.

Altenburg, L.C., Ray, J.H., Smart, C.E., Moore, F.B., 1979. Rubber solvent: a clastogenetic agent that fails to induce sister-chromatid exchanges. Mutation Research 67, 331–341.

Balague, C., Sturtz, N., Duffard, R., Evangelista de Duffard, A.M., 2001. Effect of 2,4-dichlorophenoxyacetic acid herbicide on *Escherichia coli* growth, chemical composition, and cellular envelope. Environmental Toxicology 16, 43–53.

Bianchi, N.O., Bianchi, M.S., Larramendy, M.L., 1979. Kinetics of human lymphocyte division and chromosomal radiosensitivity. Mutation Research 63, 317–324.

Duchnowicz, P., Koter, M., 2003. Damage to the erythrocyte membrane caused by chlorophenoxyacetic herbicides. Cellular and Molecular Biology Letters 8, 25–30.

EPA—Environmental Protection Agency, 1974. Compendium of Registered Pesticides. US Government Printing Office, Washington, DC.

Espandiari, P., Thomas, V.A., Glauert, H.P., O'Brien, M., Noonan, D., Robertson, L.W., 1995. The herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) is a peroxisome proliferator in rats. Fundamental and Applied Toxicology 26, 85–90.

Espandiari, P., Glauert, H.P., Lee, E.Y., Robertson, L.W., 1999. Promoting activity of the herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) in two stage hepatocarcinogenesis. International Journal of Oncology 14, 79–84.

Filkowski, J., Besplug, J., Burke, P., Kovalchuk, I., Kovalchuk, O., 2003. Genotoxicity of 2,4-D and dicamba revealed by transgenic *Arabidopsis thaliana* plants harboring recombination and point mutation markers. Mutation Research 542, 23–32.

George, T.K., Waite, D., Liber, K., Sproull, J., 2003. Toxicity of a complex mixture of atmospherically transported pesticides to *Ceriodaphnia dubia*. Environmental Monitoring and Assessment 85, 309–326.

Gibb, C., Satapanajaru, T., Comfort, S.D., Shea, P.J., 2004. Remediating dicamba-contaminated water with zerovalent iron. Chemosphere 54, 841–848.

Grossmann, K., Kwiatkowski, J., Tresch, S., 2001. Auxin herbicides induce H(2)O(2) overproduction and tissue damage in cleavers (*Galium aparine* L.). Journal of Experimental Botany 52, 1811–1816.

Gu, J., Cheng, S., Gu, J., 2001. Degradation of the herbicide dicamba under strictly anaerobic conditions. Huan Jing Ke Xue 22, 111–113.

Holland, N.T., Duramad, P., Rothman, N., Figgs, L.W., Blair, A., Hubbard, A., Smith, M.T., 2002. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. Mutation Research 521, 165–178.

Hrelia, P., Vigagni, F., Maffei, F., Morotti, M., Colacci, A., Perocco, P., Grilli, S., Cantelli-Forti, G., 1994. Genetic safety evaluation of pesticides in different short-term tests. Mutation Research 321, 219–228.

International Agency for Research on Cancer (IARC), 1991. Occupational exposures in insecticide application, and some pesticides. International Agency for Research on Cancer, Lyon.

James, L.A., Mitchell, E.L., Menansce, L., Varley, J.M., 1997. Comparative genomic hybridization of ductal carcinoma in situ of the breast: identification of regions of DNA amplification and deletion in common with invasive breast carcinoma. Oncogene 14, 1059–1065.

Lamberti, L., Bigatti-Ponzetto, P., Ardito, G., 1983. Cell kinetics and sister chromatid exchange frequency in human lymphocytes. Mutation Research 120, 193–199.

Larramendy, M.L., Reigosa, M.A., 1986. Variation in sister chromatid exchange frequencies between human and pig whole blood, plasma leukocyte, and mononuclear leukocyte cultures. Environmental Mutagenesis 8, 543–554.

Larramendy, M.L., Knuutila, S., 1990. Immunophenotype and sister chromatid differentiation: a combined methodology for analyzing cell proliferation in unfractionated lymphocyte cultures. Experimental Cell Research 188, 209–213.

Leifer, Z., Kada, T., Mandel, M., Zeiger, E., Stafford, R., Rosenkranz, H.S., 1981. An evaluation of tests using DNA repair-deficient bacteria for predicting genotoxicity and carcinogenicity. A report of the U.S. EPA's Gene-TOX Program. Mutation Research 87, 211–297.

Miller, B.M., Adler, I.D., 1989. Suspect spindle poisons: analysis of c-mitotic effects in mouse bone marrow cells. Mutagenesis 4, 208–215.

Mohammed, K.B., Ma, T.H., 1999. Tradescantia-micronucleus and -stamen hair mutation assays on genotoxicity of the gaseous and liquid forms of pesticides. Mutation Research 426, 193–199.

Norppa, H., Sorsa, M., Pfaffli, O., Vainio, H., 1980. Styrene and styrene oxide induce SCEs and are metabolised in human lymphocyte cultures. Carcinogenesis 1, 357–361.

Norppa, H., Vainio, H., Sorsa, M., 1983. Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Research 43, 3579–3582.

Norppa, H., Hirvonen, A., Järventaus, H., Uusküla, M., Tasa, G., Ojajärvi, A., Sorsa, M., 1995. Role of *GSTT1* and *OSTM1* genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. Carcinogenesis 16, 1261–1264.

Norppa, H., 2004. Cytogenetic biomarkers and genetic polymorphisms. Toxicology Letters 149, 309–334.

Peixoto, F., Vicente, J.A., Madeira, V.M., 2003. The herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) interacts with mitochondrial bioenergetic functions. Archives of Toxicology 77, 403–409.

Perocco, P., Ancora, G., Rani, P., Valenti, A.M., Mazzullo, M., Colacci, A., Grilli, S., 1990. Evaluation of genotoxic effects of the herbicide dicamba using in vivo and in vitro test systems. Environmental and Molecular Mutagenesis 15, 131–135.

Plewa, M.J., Wagner, E.D., Gentile, G.J., Gentile, J.M., 1984. An evaluation of the genotoxic properties of herbicides following plant and animal activation. Mutation Research 136, 233–245.

Ray, J.H., Altenburg, L.C., 1978. Sister chromatid exchange induction by sodium selenite: dependence on the presence of red blood cells or red blood cell lysate. Mutation Research 54, 343–354.

Roy, J.W., Hall, J.C., Parkin, G.W., Wagner-Riddle, C., Clegg, B.S., 2001. Seasonal leaching and biodegradation of dicamba in turfgrass. Journal of Environmental Quality 30, 1360–1370.

Soloneski, S., González, M., Piaggio, E., Apezteguía, M., Reigosa, M.A., Larramendy, M.L., 2001. Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. I. Genotoxic evaluation on cultured human lymphocytes exposed in vitro. Mutagenesis 16, 487–493.

Soloneski, S., Reigosa, M.A., Larramendy, M.L., 2002. Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. II. Clastogenesis on immunophenotyped human lymphocytes assessed by the micronucleus test. Environmental and Molecular Mutagenesis 40, 57–62.

Sorensen, K.C., Stucki, J.W., Warner, R.E., Wagner, E.D., Plewa, M.J., 2005. Modulation of the genotoxicity of pesticides reacted with redox-modified smectite clay. Environmental and Molecular Mutagenesis 46, 174–181.

Waters, M.D., Nesnow, S., Simmon, V.F., Mitchell, A.D., Jorgenson, T.A., Valencia, R., 1981. Pesticide Chemist and Modern Toxicology. American Chemical Society, Washington, DC.

Wiencke, J.K., Christiani, D.C., Kelsey, K.T., 1991. Bimodal distribution of sensitivity to SCE induction to diepoxybutane in human lymphocytes. I. Correlation with chromosomal aberrations. Mutation Research 248, 17–26.