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Genotoxicity of sulcotrione pesticide and photoproducts on *Allium cepa* root meristem

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ABSTRACT

Contamination by toxic agents in the environment has become matters of concern to agricultural countries. Sulcotrione, a triketone herbicide used to control dicotyledonous weeds in maize culture is rapidly photolyzed on plant foliage and generate two main photoproducts the xanthene-1,9-dione-3,4-dihydro-6-methylsulfonyl and 2-chloro-4-mesylbenzoic acid (CMBA). The aim of this study was to analyze the potential toxicity of the herbicide and the irradiated herbicide cocktail. Cytotoxicity and genotoxicity of non irradiated and irradiated sulcotrione were investigated in *Allium cepa* test. The sulcotrione irradiation was monitored under sunlight simulated conditions to reach 50% of phototransformation. Concentrations of sulcotrione in the range 5×10^{-9}–5×10^{-5} M were tested. Cytological analysis of root tips cells showed that both non irradiated and irradiated sulcotrione caused a dose-dependent decrease of mitotic index with higher cytotoxicity for the irradiated herbicide which can lead to 24.2% reduction of mitotic index compared to water control. Concomitantly, chromosomal aberrations were observed in *A. cepa* root meristems. Both non irradiated sulcotrione and irradiated sulcotrione induced a dose-dependent increase of chromosomal abnormalities frequencies to a maximal value of 33.7%. A saturating effect in anomaly frequencies was observed in meristems treated with high concentrations of non irradiated sulcotrione only. These data suggest that photolyzed sulcotrione cocktail have a greater cytotoxicity and genotoxicity than parent molecule and question about the impact of photochemical process on environment.

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1. Introduction

Chemical pollutants reveal increasing threats to environment, amplified by population growth and climate change. Humans and ecosystems are exposed to highly variable and unknown cocktail of chemicals. Although individual chemicals are typically present at low concentrations, they can interact with each other resulting in additive or potentially synergistic mixture effects. The concomitant presence of various pesticides and their transformation products adds further complexity to chemical risk assessment [1]. Recent studies have proved that exposure to sunlight can be one of the most destructive factors for pesticides following the crop treatment [2]. Therefore, a fast herbicide degradation can reduce crop treatment efficiencies [3] and generate by-products [4] in the environment that can play a role in specifying the impact of pesticides on both natural ecosystems and human health [5–7].

2-(2-Chloro-(4-methylsulfonyl)benzoyl)-1,3-cyclohexanedione also named sulcotrione, belongs to the class of triketone herbicides used in maize cultures and was proposed as atrazine substitute [8]. In target plant species, the action of sulcotrione consists in the inhibition of the enzyme 4-hydroxyphenyl pyruvate dioxygenase (HPPD) [9,10] leading to strong bleaching effects accompanied by a decrease in chlorophyll and carotenoid levels as well as by a massive accumulation of phytoene [11,12], necrosis and death of sensitive plants. Sulcotrione is absorbed by the leaves and or the root system [13,14] and may accumulate in the soil more than a month after application [13–15]. Water solubility of the product is 165 mg/L at 25° C with a great potential to leach [13,14]. It was assumed that maize population density was 100,000 plants per ha and application rate amounts to 300 g of sulcotrione per ha [16]. It corresponds to a concentration of 3 mg of active substance per plant (or 30 mg/m^2).

Although sulcotrione is used in crop protection scheme since 1993, its environmental fate is still poorly documented. In soils its major transformation product has been identified as 2-chloro-4-mesylbenzoic acid (CMBA) [14]. Sulcotrione absorbs in actinic part of solar light and undergoes direct photodegradation. The irradiated sulcotrione mainly gives a cyclization product (xanthene-1,9-dione-3,4-dihydro-6-methylsulfonyl), and CMBA formed in

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smaller amounts depending on pH of the solution [17]. These two photoproducts had no herbicide activity and present toxicity towards unicellular organisms different of sulcotrione toxicity [16,18]. For bacteria and protozoan, the cyclization product was more toxic than the parent molecule suggesting that this photoproduct should be considered in sulcotrione environmental risk assessment [4,19]. It has clearly been demonstrated that the cyclization product (xanthene) is the main product formed and potential toxicity of this major product has been suggested [18]. In case of sulcotrione, oral administration to rats significantly inhibited hepatic HPPD [20].

Higher plants provide a useful genetic system for screening and monitoring environmental pollutants. Meristematic mitotic cells are appropriate indicator cells for the detection of genotoxicity. *Allium* and *Vicia* were shown as efficient test systems for root micronuclei and chromosomal aberrations [21]. Cytological analysis of *Vicia* root tips cells have previously shown aneugenic effects of sulcotrione. It induced chromosomal alterations at low concentration (10^{-5} M) indicating potent mutagenic effect of the herbicide. Moreover, sulcotrione is persistent for a short time but mobile in soil [13–15,22].

The present study establishes genotoxicity of sulcotrione on *Allium cepa* using cytogenetic bioassays by testing the changes of mitotic index (MI), and the frequencies of abnormal chromosomes. Potential genotoxic effects of the herbicide on *Allium* was compared to the one generated by irradiated sulcotrione solutions. This is the first study that reports the photochemistry impact on pesticide induced-genotoxicity.

2. Materials and methods

2.1. Irradiation conditions

Aqueous sulcotrione (2×10^{-4} M) was prepared in purified water and pH was adjusted to 2 to accelerate sulcotrione photodegradation rate. Sunlight-simulated irradiations were performed in a Suntest CPS photosimulator (Atlas). A 500 Wm^{-2} surface energy was used to simulate the sunlight average intensity received in June in France. The light intensity emitted by lamp within the wavelength range 290–420 nm was measured using a radiometer QE65000 from Ocean optics. The internal temperature was maintained at approximately 35 ± 2 °C with cooled water (15 °C) flowing through the bottom of the sample holder. Sulcotrione (200 mL) was irradiated in a 600 mL glass beaker reactor using a device equipped with an air cooling system.

2.2. Analytical equipment

UPLC-UV analyses were carried out at room temperature using a photodiode array detector chromatograph coupled with a reverse phase column (HSS T3 1.8 µm, 2.1 × 100 mm, Waters). A flow rate of 0.5 $mL.min^{-1}$ was used for all analyses. The gradient consisted of a mixture of 25% acetonitrile and 75% water (acidified with formic acid, pH 2.5). After 1 min, the proportion was linearly increased to 50% acetonitrile within 9 min in order to monitor chromatographic separation of sulcotrione and its photoproducts. Detection was performed at 284 and 243 nm. At pH 2.5 the molar absorption coefficients are of 5500 $mol^{-1}.L.cm^{-1}$, 27,300 $mol^{-1}.L.cm^{-1}$, and 12,000 $mol^{-1}.L.cm^{-1}$ for CMBA, xanthene and sulcotrione, respectively.

2.3. Plant assay system

Bulbs of onion (*A. cepa L. var. aggregatum*) were purchased at local store. Bulbs were long-half traditional shallot, 24–44 caliber class 1, Bretagne origin. Pink shallots were "Jersey" variety and cultivated from a biological culture. Root tip cells of *A. cepa* ($2n = 16$) were used as test system and both sulcotrione (Sigma–Aldrich, France) and irradiated sulcotrione cocktail as test substances. Healthy bulbs were placed in small jars of 40 ml with basal ends dipping in distilled water and germinating in darkroom at room temperature (25 °C ± 2 °C). When the newly emerged roots were 1–2 cm in length, they were washed and used in experiments. Roots of *Allium* were treated with series of concentrations of non irradiated sulcotrione and irradiated sulcotrione. Treatments used irradiated samples diluted to reach concentrations of sulcotrione in the range 5×10^{-9}–5×10^{-5} M. It is considered as the maximal concentration of sulcotrione to represent 3 mg of sulcotrione (the quantity for one plant) in 40 mL (the capacity of one flask). Concomitantly, a non irradiated sulcotrione treatment was made with the same sulcotrione concentration as the irradiated sulcotrione. All chemical treatments were conducted in 25 mM phosphate buffer pH 6.8 in order to neutralize sulcotrione solutions. Negative control was conducted in phosphate buffer and maleic hydrazide (4×10^{-3} M) was used as positive controls. Experiments were conducted for 48 h at room temperature 25 ± 1 °C in a darkroom.

2.4. Cytotoxicity and genotoxicity tests

Excised root tips were fixed for 24 h in Clarke's solution (ethanol 99% and glacial acetic acid 3:1) and then stored in 70% ethanol at 4 °C. Root tips were hydrolyzed with 1 N HCl for 5 min and incubated in acetic-orcein (1%) for at least 30 min. Root tips were then squashed in 45% acetic-acid on slides and examined under an Olympus microscope. 3–5 bulbs were tested for each concentration of non irradiated or irradiated treatment. 6–8 roots were examined separately for each bulb. The analysis of the mitotic index was made in random fields (≈0.2 mm^2). An average of 500 cells was scored from each different root to get a total of 3000 cells for one bulb. MI was calculated from the number of dividing cells/total number of cells × 100. Chromosome aberrations i.e. chromatin bridges, stickiness, stars, laggard, vagrant chromosome and fragments were characterized in anaphase and telophase cells. All anaphase and telophase cells were accounting in the whole meristem of each root. Only roots with at least 20 anaphases and telophases were considered for the study. Chromosomal abnormality frequency was calculated from the number of aberrant cells/Anaphase and Telophase cells × 100.

All experiments were performed in triplicate and repeated at least once. Each sample was encoded by another researcher in order to not influence the second one for determination of treatment genotoxicity.

2.5. Statistical analysis

Data from non irradiated sulcotrione and irradiated sulcotrione were compared using one way ANOVA analysis and Tukey HSD All-Pairwise Comparisons Test and correlated with Statistix 10.0 software. Differences between each group of abnormality were established using non parametric Kruskal–Wallis Analysis of Variance on Ranks analysis and Dunn's Test.

3. Results

3.1. Preparation of the irradiated sulcotrione cocktail

Substances used in plant bioassay system were the parent molecule (non irradiated sulcotrione) and irradiated sulcotrione cocktail. The cocktail corresponded to sulcotrione photodegraded in controlled conditions to a conversion extent of 50%. After a half life

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