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# UV–vis degradation of iprodione and estimation of the acute toxicity of its photodegradation products

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ABSTRACT

The UV–vis photodegradation of iprodione in water was investigated with a high pressure mercury lamp photoreactor. Five photoproducts of iprodione were characterized by LC–HR-MS/MS and isotopic labeling; none of them has been reported in previous studies. Three of them result from the elimination of one or two chlorine atoms followed by hydroxy or hydrogen addition while the two others are cyclic isomers of iprodione. An ICR mass spectrometer was used for by-products identification; concentrations of photoproducts were estimated with a triple quadrupole instrument, using iprodione-$D_5$ as an internal standard. Phototransformation mechanisms were postulated to rationalize photoproducts formation. *In silico* QSAR toxicity predictions were conducted with the Toxicity Estimation Software Tool (T.E.S.T.) considering oral rat LD50, mutagenicity and developmental toxicity. Low oral rat LD50 values of 350 mg/kg and 759 mg/kg were predicted for cyclic isomers of iprodione, compared to that of the parent molecule (2776 mg/kg). Toxicity estimations exhibited that all the iprodione photoproducts could be mutagenic while the parent compound is not. *In vitro* assays on *Vibrio fischeri* were achieved on both irradiated and non-irradiated aqueous solutions of iprodione and on HPLC fractions containing isolated photoproducts. Phenolic photoproducts were shown to be mainly responsible for toxicity enhancement with EC50 values of 0.3 and 0.5 ppm, for the bi- and mono-phenolic compounds issued from chlorine elimination.



## 1. Introduction

Pesticide usage is steadily increasing every year throughout the world. The Environmental Protection Agency estimates indicate a growth of 25% for the fungicides market size between 1998 and 2007 and the involved amounts raised the scientific community awareness on these molecules toxicity and stability [1]. Among these, iprodione (3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide) is a dicarboximide fungicide. Known as "Rovral", it was first introduced to the market in 1974 by Rhône-Poulenc [2]. It is mainly applied to prevent gray mold for ornamental cultivations [3] but also on crops, such as vineyards [4–6], apple trees [7] and tomato plants [8,9]. Its wide range of applications also includes golf greens protection [10]. It is used to protect crops from a wide range of pathogenic fungi such as *Fusarium*, *Botrytis* or *Phytophtora* [11]. Unlike most of the fungicides of this family, iprodione does not appear as an AR (Androgen Receptor) antagonist but rather as an inhibitor of steroidogenesis [12]. It alters adrenal gland function and induces interstitial cell tumors in the rat testis [13]. More recent studies have shown that iprodione could delay pubertal development in male rats [12]. According to a review of the European Commission of 2002, iprodione could be responsible for short term and long term effects such as atrophy, hyperplasia and weight change on the liver, ovary, kidney, seminal vesicles [14]. There are few data available about ecotoxicological effects of iprodione on marine organisms; they describe the effect of fungicides on aquatic fungi and microorganisms constituting interesting biomarkers [15,16]. In addition, iprodione has the capacity to diffuse in water and then is likely to cause damage on these organisms. Furthermore, Radice et al. reported that iprodione was able to produce oxidative damage in primary cultured fish hepatocytes at the concentrations of 0.3 and 0.4 mM [15]. In the agricultural field, the elimination of pesticides results from biological, chemical and/or photochemical degradation. The study of the photochemical behavior of these pollutants is thus a key element for the understanding of toxics formation [17,18]. Iprodione acts as a contact fungicide and only a fraction of the applied product penetrates the plant cuticle, thus making it very

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prone to receive long-term sunlight irradiation [19,20]. The curative or preventive effects of fungicides are not only based on their mechanism of action but also on their chemical properties such as solubility in water or log $K_{ow}$, which will define which part of the plant they will target [21,22]. Photolysis can occur directly on the plant but also on the soil, and the media will greatly impact chemical processes [17]. The dicarboximide family has been previously known to be susceptible to photocatalytic and photolytic degradation. Hustert et al. described the photodegradation of vinclozolin and procymidone catalyzed by semiconductors such as iron oxide and titanium dioxide [23]. Schick et al. proposed photodegradation pathways for vinclozonin in water and methanol–water solutions [24]. More recently, the photodegradation of procymidone was examined by Rifai et al. along with the potential toxicities of formed products using *in silico* tests [25]. Iprodione has been detected after application on crops in several studies [5,26]. No study so far has reported the quantitation of photoproducts after the application of iprodione on crops. The main objective of this work was the identification of the transformation products issued from direct photolysis of iprodione in water, in order to simulate photolysis conditions on fruits or leaves after application. It is now established that liquid chromatography coupled with high resolution mass spectrometry is a method of choice for the identification of unknown contaminants in water or food [27–29]. The analytical method preferred in this work was the combination of liquid chromatography with tandem mass spectrometry (LC–MS/MS) using a high resolution FT-ICR to detect and help identifying former photoproducts. The second goal was the evaluation of the potential toxicities of these photoproducts using *in silico* QSAR (Quantitative Structure–Activity Relationship) calculations. Finally fraction separation was achieved for each photoproduct in order to assess *in vitro* toxicities on *Vibrio fischeri* with photoluminescence comparisons.

## 2. Experimental

### 2.1. Chemicals and reagents

Iprodione (99% purity) and iprodione-$D_5$ were purchased from Sigma Aldrich (St Quentin Fallavier, France) and Cluzeau Info Lab (Sainte Foy La Grande, France, respectively). Their chemical structures are displayed in Fig. 1. Chromatographic grade solvents (99.99% purity), acetonitrile (ACN) and formic acid (FA), were also purchased from Sigma Aldrich. Considering the poor solubility of iprodione in water (13.9 mg/L at 25 °C) [30], solutions of iprodione and iprodione-$D_5$ at 100 mg/L was prepared in an ACN/water 30:70 mixture. ACN does not absorb light in the UV range (0% at 200 nm) [31]. All solutions were degased using nitrogen bubbling for 15 min and sonication for 10 min. A constant pH value of 5.5 was measured at 0, 90 and 180 min.

### 2.2. Photolysis experiments

Photolysis experiments were carried out using a self-made reactor equipped with a high-pressure mercury lamp (HPL-N 125W/542 E27 SC; Phillips, Ivry-sur-Seine, France) delivering radiation at wavelengths ranging from 200 nm to 650 nm. According to manufacturer data, the incident radiation flux was 6200 lm. The reactor consists in six quartz tubes of 120 mL disposed in a circle around the lamp and immerged into a sonicator (AL04-12, Advantage-Lab, Switzerland) filled with deionized water. During experiments, the reactor was regularly cooled by water circulation to avoid uncontrolled heating of the irradiated solutions and to maintain a constant temperature of 25 ± 3 °C. For each experiment, 60 mL of a solution of iprodione (see above) were used. To follow the kinetic evolution of photoproducts, a series of experiments was carried out with 25 irradiation times ranging from 0 to 180 min: 0, 2, 4, 6, 8, 10, 12, 15, 18, 22, 26, 30, 40, 50, 55, 60, 70, 80, 90, 100, 110, 120, 140, 160 and 180 min. All the irradiated solutions were analyzed by LC–MS. A reference solution of iprodione was degased, sonicated and kept 180 min at 25 °C without being submitted to irradiation. Analyses showed that iprodione did not undergo any degradation under these conditions.

### 2.3. LC–MS operating conditions

All the chromatographic separations were carried out on a liquid chromatography Acquity HPLC system (Waters Technologies, Saint Quentin en Yvelines, France). 10 μL of the sample were injected and separated on a $C_{18}$ Poursuit XRs$^{Ultra}$ 2.8 μm 100 mm × 2.0 mm column (Agilent Technologies, Les Ulis, France). Elution was performed using a 0.2 mL/min solvent flow with a gradient increasing from 40% of B solvent to 95% of B solvent in 25 min (A: water + 0.1% formic acid; B: acetonitrile + 0.1% formic acid). The gradient was then set at 40% of B solvent for the last 10 min. For structural elucidation experiments, the HPLC system was coupled with a high resolution Solarix FT-ICR 7T mass spectrometer (Bruker Daltonics, Bremen, Germany). A 1:60 split was set between the column and the ESI nebulizer in order to get a flow of 200 μl/h in the FT-ICR source. An electrospray ion source was used in the positive mode. Ions were accumulated 0.01 s in the source hexapole and 0.2 s in the collision cell with a cooling time of 0.01 s. Time of flight in the optic transfer was set at 0.5 ms with a 0.1 s dwell time. The detection parameters were set using a resolution of 512,000 pts to record ions on the *m/z* 100 to *m/z* 500 mass range, with a 0.1835 ms transient duration in the broadband mode. Four acquired scans were averaged for each spectrum, corresponding to MS or MS/MS duty cycles of approximately 1.6 s. In MS/MS experiments, the precursor ion was selected in the quadrupole with an isolation window of 4 Da and submitted to collision induced dissociation with a collision energy of 8 V. For quantitation experiments, the same HPLC system was coupled with a MS-6410 triple quadrupole (TQ) mass spectrometer (Agilent Technologies, Les Ulis, France). Chromatographic conditions were the same as those described above. The capillary, ESI cone and ion guide voltages were set at 5 kV, 600 V and 20 V, respectively. Source and desolvation temperatures were respectively fixed at 50 °C and 300 °C. Nitrogen was used for nebulization and desolvation at pressures of 16 psi and 57 psi, respectively. Given that experiments were performed in pure water and that neither

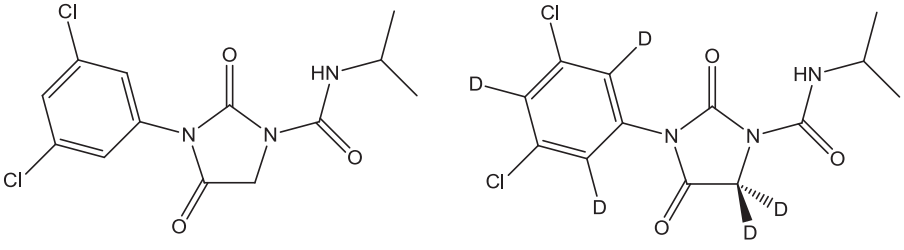


**Fig. 1.** Chemical structures of iprodione and iprodione-$D_5$.

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