Occurrence of 2,4-Dichlorophenol and of 2,4-Dichloro-6-Nitrophenol in the Rhône River Delta (Southern France)

3 AUTHORS:

Serge Chiron
Université de Montpellier
87 PUBLICATIONS **2,628** CITATIONS

SEE PROFILE

Claudio Minero
Università degli Studi di Torino
329 PUBLICATIONS **8,151** CITATIONS

SEE PROFILE

Davide Vione
Università degli Studi di Torino
265 PUBLICATIONS **3,755** CITATIONS

SEE PROFILE

Occurrence of 2,4-Dichlorophenol and of 2,4-Dichloro-6-Nitrophenol in the Rhône River Delta (Southern France)

SERGE CHIRON,[†]
CLAUDIO MINERO,[‡] AND
DAVIDE VIONE*,[‡]

Laboratoire Chimie et Environnement, Université de Provence, 3 place Victor Hugo, 13331 Marseille cedex 3, France and Dipartimento di Chimica Analitica, Universitá di Torino, Via Pietro Giuria 5, 10125 Torino, Italy.

The compounds 2,4-dichlorophenol (2,4-DCP) and 2,4-dichloro-6-nitrophenol (6-nitro-2,4-DCP) have been detected at μg L^{-1} levels (10^{-9}–10^{-8} M) during the summer season 2005 in the water of the Rhône river delta. Compound 2,4-DCP would mainly derive from the transformation of the herbicide dichlorprop, heavily used in flooded rice farming (1400 kg in the delta region in 2005), in addition to being an impurity of the commercial herbicide. Field data show a fast concentration decrease of 2,4-DCP in the period June 21st to July 5th, accompanied by a corresponding increase of 6-nitro-2,4-DCP. This could imply a possible nitration process of 2,4-DCP into 6-nitro-2,4-DCP, with quite elevated yield (33%). Nitration of 2,4-DCP can be induced by photoproduced $^\bullet$NO$_2$, the reaction kinetics (calculated in the presence of Fe(III) + nitrite under irradiation as model system) being d[6-nitro-2,4-DCP]/dt = 650 [2,4-DCP] [$^\bullet$NO$_2$]. Interestingly, the yield of the process (38%) is similar to that suggested by field data. An indirect assessment of [$^\bullet$NO$_2$] in surface water in different sites of the Rhône delta indicated that 2,4-DCP could be transformed into 6-nitro-2,4-DCP in a couple of weeks or less in the shallow water (10 cm depth) of the rice fields, a time scale that is compatible with field data. Photonitration of 2,4-DCP is thus a possible process to account for the occurrence of 6-nitro-2,4-DCP in the Rhône delta.

Introduction

The compound 2,4-Dichlorophenol (2,4-DCP) is a toxic chlorinated compound that can be found at concerning concentration levels in surface water and sediment (*1, 2*). It can originate from the environmental transformation of herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4-dichloro-phenoxy)propionic acid (dichlorprop) (*3, 4*), and of the anti-microbial agent 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan) (*5, 6*).

Compound 2,4-DCP is cytotoxic toward freshwater and marine algae (*7, 8*), and animal cells and tissues (*4, 9*). It has been shown to alter the activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase) (*10*), and fish

exposure to this compound is actually associated with disorder in antioxidant defenses and lipid peroxidation (*11*). Environmental transformation processes of 2,4-DCP might involve microbial and photochemical degradation or dark oxidation by Mn oxides, and in all such cases oxidation and dechlorination have been observed (*12–15*). In the present paper, we will demonstrate that 2,4-DCP can undergo nitration to 2,4-dichloro-6-nitrophenol (6-nitro-2,4-DCP) under laboratory conditions, and we will give evidence that nitration could also occur in the environment.

Compound 6-Nitro-2,4-DCP is used as an herbicide in some countries but it is not commercialized in France. It differs from 2,4-DCP in that can induce gene mutations and chromosomal aberrations (*16, 17*) and acts as inhibitor of phenol sulfotransferase (*18*), which belongs to a class of enzymes that are involved in the detoxification of xenobiotics (*19*). The genotoxicity of 6-nitro-2,4-DCP is a typical feature of many aromatic nitroderivatives that can form covalent adducts with DNA or induce oxidative DNA damage on their enzymatic reduction to the corresponding amines (*20*).

As far as phototransformation is concerned, note that 2,4-DCP shows an absorption tail in the UVB region, absorption by the corresponding phenolate anion approaches the UVA, while 6-nitro-2,4-DCP absorbs radiation below 540 nm (also see Figure C in the Supporting Information).

This work presents the results of a field study of 2,4-DCP and 6-nitro-2,4-DCP in the water of the Rhône delta (S. France). Data suggesting the environmental transformation of 2,4-DCP into 6-nitro-2,4-DCP are reported, and an hypothesis of the involved pathways is also presented. Laboratory photonitration studies were carried out in the presence of nitrite and Fe(III) oxide colloids, a very suitable model system although its importance in the field could be difficult to assess. Oxides have been found to constitute 40–100% of colloidal Fe in oceanic water, the remainder being accounted for by complexes with organic matter (*21*). To our knowledge, this is the first time that field evidence of an aromatic nitration (and possibly photonitration) process in surface waters was obtained, and the finding might have implications for the assessment of the health impact of pesticide metabolites. Also note that the Rhône delta region (Camargue) is a zone of great environmental importance as it is one of the main wintering sites for waterbirds in the Mediterranean region, but it is also subject to a good level of environmental stress because of agricultural activities (*22*).

Experimental Section

Reagents and Materials. See the Supporting Information (hereafter SI).

Study Area and Sampling. The schematic representation of the sampling area is illustrated in Figure A (SI). The Rhône river delta and its lagoon system are located in the southern part of France. The lagoon system (422 km²) can be divided into two sub-systems: the Vaccarès lagoon and the lower lagoons (Etang du Lion (EL) and Etang de l'Impérial (EI)) which are directly connected to the sea. The Rhône delta is devoted to intensive flooded rice cultivation. In the northern section of the delta (310 km²), irrigation water is pumped from the Rhône river and drainage water is returned to the river through a main ditch in Albaron (sampling point D1). The eastern section (87 km²) of the delta naturally drains into the Vaccarès lagoon by a low slope ditch (sampling point D2). The concentrations of 2,4-DCP and 6-nitro-2,4-DCP were also measured in the lagoon system (sampling points L1 and L2) and in the river waters at the entrance of the delta (sampling point S1) during the summer season 2005. Samples

* Corresponding author phone: +39-011-6707633; fax: +39-011-6707615; e-mail: davide.vione@unito.it.
† Université de Provence.
‡ Universitá di Torino.

were collected with a stainless steel sampling bottle. At each site, a composite water sample was collected from approximately 4−5 vertical profiles. Composite samples were filtered through 0.45 μm filters (cellulose acetate, Millipore) and subsequently split into pre-cleaned 500 mL glass bottles prepared in duplicate and stored at 4 °C until analysis. Duplicate samples were used for backup purposes in case of breakage of the primary sample and for laboratory replicates. Two precipitation sampling stations were operated in the Rhône river delta. One station (R1) was located in the northern part of the delta and the second (R2) was in the eastern part. Both stations were surrounded by paddy fields and close to a meteorological station. The rain collectors consisted of a 0.78 m^2 glass funnel coated with a Teflon foil connected to a 2.5 L glass bottle. The funnel was manually uncovered and rinsed with deionized water and methanol just before each rain event started. The bottles had been previously heated to 450 °C for 8 h. Three major rain events were sampled from April to September 2005 (April 19th, May 20th, and June 21st). Rain samples were refrigerated and filtered through 0.45 μm cellulose acetate filters immediately after collection to avoid nitrophenol biodegradation (23).

Analytical Procedures. Nitrate and nitrite ions were determined by ion chromatography (detection limit 10^{-6} M), HCO_3^-/CO_3^{2-} as inorganic carbon with a Shimadzu TOC-5000 total organic carbon analyzer, non purgeable organic carbon (NPOC) with the same instrument upon sample acidification with $HClO_4$ and 20 min purge with zero-grade air to eliminate CO_2, total iron by furnace absorption spectrophotometry. For 2,4-DCP and 6-nitro-2,4-DCP analysis, sample pH was adjusted to a value of 5 and 500 mL samples were preconcentrated with solid-phase extraction (SPE) on Oasis HLB 500 mg cartridges (Waters). The analytes were eluted with 2 × 5 mL methanol. The extract was evaporated to dryness and then redissolved in 200 μL of a water/methanol mixture (50:50 v/v). The analysis of the extract was performed by LC-MS/MS using an atmospheric pressure chemical ionization (APCI) interface in negative ionization mode as recommended for chloro- and nitrophenols (24). The most abundant product ion in MS^2 mode was chosen for LC-MS/MS analysis. The full scan MS^2 spectrum of 2,4-DCP was characterized by an intensive fragment at m/z 125 corresponding to a HCl loss while the MS^2 spectrum of 6-nitro-2,4-DCP revealed a fragment ion at m/z 176 ($[M-H-NO]^-$) as base peak. The HPLC system consisted of a LiChrospher RP-18 column 250 mm × 4.6 mm i.d., 5 μm particle size. The mobile phase used in chromatographic separation consisted of a binary mixture of solvents A (methanol) and B (water) at a flow rate of 0.8 mL min^{-1}. The gradient was operated from 40 to 100% A for 30 min and than back to the initial conditions in 5 min. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode with unit mass resolution. The selected transition ions were 161 > 125, 206 > 176, and 222 > 164 for 2,4-DCP, 6-nitro-2,4-DCP, and 2,4-D d$_3$, respectively. A typical MRM chromatogram obtained after preconcentration of 500 mL of surface water is shown in Figure B (SI). Identification of the target analytes in unknown samples was based on the LC retention time compared to that of a standard (±30 s) and the unique combination of a precursor-product ion. The percent recovery was 82 ± 8% and 96 ± 6% for 2,4-DCP and 6-nitro-2,4-DCP, respectively. Limits of quantification (LOQs) were 11 and 1 ng L^{-1}, respectively. Quantification was carried out by means of an isotope-labeled internal standard (2,4-D d$_3$) procedure because this is the only effective way to overcome the matrix ion suppression effects in LC-MS/MS (25). Calibration curves were constructed by plotting the ratio peak area/internal standard peak area against concentration levels. This ratio remained constant whether the matrix was present or not.

Irradiation Experiments. Experiments were carried out with a 0.5 L cylindrical immersion-type photoreactor (Heraeus TQ 150 model, radiation path length 2 cm), equipped with a water-cooled, medium-pressure mercury lamp with maximum emission wavelengths at 313, 366, 406, 436, 546, and 578 nm (see SI, Figure C). The reactor is made of Pyrex glass to cut off the wavelengths shorter than 290 nm. The whole assembly was mounted on a magnetic stirrer and wrapped with aluminum foil. Initial pH value was adjusted by dropwise addition of either 0.1 M NaOH or 0.1 M H_2SO_4. Note that, when mixing 2,4-DCP and $NaNO_2$, a thermal reaction occurs because 2,4-DCP is a weak acid able to protonate nitrite to HNO_2, and leading to nitroderivative formation. To avoid dark nitration to proceed before starting irradiation in such cases, the required amount of NaOH for final pH adjustment to 7 was added to the 2,4-DCP solution in one time before addition of $NaNO_2$. At pH 7 the dark reaction is no longer operational. Aliquots of 1 mL were analyzed at selected intervals after a filtration step through 0.45 μm filter membranes (cellulose acetate, Millipore). Byproduct identification was carried out by HPLC-MS^2 using an electrospray interface in negative ionization mode. The HPLC system consisted of a Metachem C-18 column 150 × 2 mm i.d., 3 μm particle size (Varian), and an Esquire 6000 ion trap mass spectrometer (Bruker, Bremen, Germany). The mobile phase used in chromatographic separation consisted of a binary mixture of solvents A (methanol) and B (0.07% formic acid solution) at a flow rate of 0.2 mL min^{-1}. The gradient was operated from 5 to 100% A for 30 min and then back to the initial conditions in 5 min. Absorption spectra were measured with a Varian Cary 100 Scan UV−vis spectrophotometer. The initial degradation rate of 2,4-DCP and the initial formation rate of 6-nitro-2,4-DCP were calculated as reported in ref 26.

Results and Discussion

Field Observations. Table 1 summarizes the results (in μg L^{-1}) from rainwater and surface water samples analyzed for 2,4-DCP and 6-nitro-2,4-DCP from April to September 2005. In addition, the amount of precipitation during this period is presented. In the Rhône river delta, 2,4-DCP mainly originated from the degradation of 2-(2,4-dichloro-phenoxy)-propionic acid (dichlorprop), a post emergent herbicide heavily used for rice farming (1400 kg in the delta region in 2005) (22). The detected dichlorprop levels, determined as described in ref 22, are also reported in Table 1. Dichlorprop is field sprayed between mid-May and mid-June. Additionally, 2,4-DCP can make up more than 8% of the commercial herbicide (27), and this impurity was probably another source of 2,4-DCP input into the delta waters. The compound 2,4-DCP was primarily detected right after the main application period and, for instance, it peaked at a level of 4.72 μg/L on June 21st at the outlet of a major ditch (D1) which drained the paddy fields into the river. Generally, there was a certain trend in dilution into the lagoons with a maximum concentration value of 2,4-DCP recorded at 0.23 μg/L on June 21st. These relatively high concentration values fell rapidly to a maximum value of 0.04 μg/L recorded in late September in the lagoon. Simultaneously to the decrease of 2,4-DCP, a steady increase of 6-nitro-2,4-DCP was observed. Peak concentrations of 6-nitro-2,4-DCP were observed with a delay of two weeks with respect to 2,4-DCP. Over the sampling period, the 6-nitro-2,4-DCP/2,4-DCP ratio changed markedly from 0.28 on June 21st to 2.8 on July 5th, suggesting a likely transformation of 2,4-DCP into 6-nitro-2,4-DCP. The time trend of 2,4-DCP and of 6-nitro-2,4-DCP in sampling site D1 is reported in Figure 1 (molar concentration values).

In an attempt to assess the possible contribution of precipitation to surface water levels, measurements of 6-nitro-2,4-DCP and of 2,4-DCP were also carried out in rain

TABLE 1. Analytical Data of 2,4-DCP and 6-nitro-2,4-DCP in the Different Sampling Sites and Dates[a]

compound	sampling date	D1	D2	L1	L2	R1	R2	S1	precipitation amount (mm)
dichlorprop (μg L^{-1})	19 April 05	n.d	n.d	n.d	n.d	n.i	n.i	n.d	69.1
	20 May 05	$0.04 \pm 6 \times 10^{-3}$	n.d	n.d	n.d	n.i	n.i	n.d	97.2
	21 June 05	3.11 ± 0.37	0.71 ± 0.09	0.11 ± 0.01	0.08 ± 0.01	n.i	n.i	n.d	13.9
	5 Jul05	0.41 ± 0.05	0.18 ± 0.02	$0.06 \pm 7 \times 10^{-3}$	n.d	n.i	n.i	n.d	0
	18July 05	n.d	$0.05 \pm 6 \times 10^{-3}$	n.d	n.d	n.i	n.i	n.d	0
	24 August 05	n.d	n.d	n.d	n.d	n.i	n.i	n.d	0
	22 September 05	n.d	n.d	n.d	n.d	n.i	n.i	n.d	0
2,4-DCP (μg L^{-1})	19 April 05	$0.05 \pm 5 \times 10^{-3}$	n.d	$0.02 \pm 3 \times 10^{-3}$	$0.015 \pm 2 \times 10^{-3}$	0.16 ± 0.024	0.11 ± 0.017	n.d	69.1
	20 May 05	0.12 ± 0.02	0.07 ± 0.01	0.015 ± 0.002	n.d	$0.04 \pm 6 \times 10^{-3}$	$0.03 \pm 4 \times 10^{-3}$	n.d	97.2
	21 June 05	4.72 ± 0.71	1.23 ± 0.18	0.18 ± 0.03	0.23 ± 0.035	$0.02 \pm 3 \times 10^{-3}$	n.d	n.d	13.9
	5 Jul05	0.85 ± 0.13	0.42 ± 0.06	$0.06 \pm 9 \times 10^{-3}$	$0.04 \pm 6 \times 10^{-3}$	n.d	n.d	n.d	0
	18July 05	0.33 ± 0.05	0.18 ± 0.03	n.d	n.d	n.d	n.d	n.d	0
	24 August 05	$0.04 \pm 6 \times 10^{-3}$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	0
	22 September 05	$0.03 \pm 5 \times 10^{-3}$	n.d.	$0.04 \pm 6 \times 10^{-3}$	n.d.	n.d.	n.d.	n.d	0
6-nitro- 2,4-DCP (μg L^{-1})	19 April 05	n.d.	n.d.	n.d.	n.d.	$0.06 \pm 5 \times 10^{-3}$	n.d.	n.d	69.1
	20 May 05	$0.06 \pm 5 \times 10^{-3}$	n.d	n.d	n.d	0.12 ± 0.01	$0.08 \pm 7 \times 10^{-3}$	n.d	97.2
	21 June 05	1.32 ± 0.12	0.44 ± 0.04	0.16 ± 0.015	0.19 ± 0.03	$0.02 \pm 2 \times 10^{-3}$	n.d.	n.d	13.9
	5 July 05	2.46 ± 0.22	1.12 ± 0.11	0.31 ± 0.03	0.42 ± 0.04	n.d.	n.d.	n.d	0
	18 July 05	1.24 ± 0.11	0.92 ± 0.08	0.23 ± 0.02	0.28 ± 0.025	n.d	n.d	n.d	0
	24 August 05	0.90 ± 0.08	0.63 ± 0.06	0.11 ± 0.01	0.14 ± 0.01	n.d.	n.d	n.d	0
	22 September 05	0.45 ± 0.04	0.12 ± 0.01	$0.07 \pm 6 \times 10^{-3}$	n.d	n.d	n.d.	n.d	0

[a] D1, D2 are ditches; L1, L2 are lagoons; R1, R2 are rainwater sampling stations; S1 is the sampling station at the inlet of the delta. See Figure A (SI) for the location of each sampling site. Error bounds: ± 1 standard deviation of triplicate runs. The dichlorprop data obtained in the same period are also reported. n.d is not determined (below detection limit). n.i is not investigated.
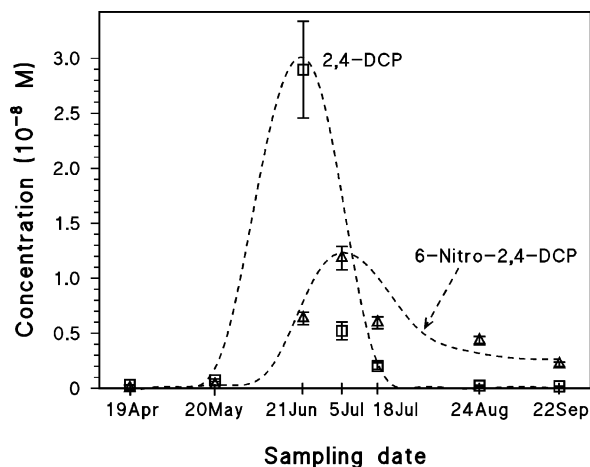

FIGURE 1. Time trend of 2,4-DCP and of 6-nitro-2,4-DCP in sampling site D1 (see Figure A in the SI for the site location). The *x* axis reports the exact sampling dates. A similar time trend (2,4-DCP maximum on June 21st, 6-nitro-2,4-DCP maximum on July 5th) was also observed in the other sampling sites in the Rhône Delta (D2, L1, and L2, see Table 1).

samples collected during the three rain events occurred through the sampling period. A significant drop in concen-

tration in the first few millimeters of rain can be expected for such chemicals. This hypothesis was tested by sequential sampling of rainwater from a single rain event. Concentrations were found to be maximal (0.16 and 0.12 μg/L for 2,4-DCP and 6-nitro-2,4-DCP, respectively) during the first 10 mm of the rain event and rapidly decreased by a factor of 10 within the following 10 mm. The washout of 2,4-DCP and 6-nitro-2,4-DCP underwent a so-called first flush effect as already reported in the case of various pesticides (28). Consequently, rain events might prompt a very limited load of 2,4-DCP and 6-nitro-2,4-DCP into surface waters. In addition, 2,4-DCP and 6-nitro-2,4-DCP were not found at the entrance of the Rhône delta (sampling site S1), indicating that they were not transported by the Rhône water dissolved phase. An additional pathway for the occurrence of 6-nitro-2,4-DCP could be volatilization of the more volatile 2,4-DCP, followed by gas-phase nitration into 6-nitro-2,4-DCP by $^{\bullet}$OH + $^{\bullet}$NO$_2$ or $^{\bullet}$NO$_3$ + $^{\bullet}$NO$_2$ (29), and final dry deposition of the nitroderivative back into the water phase. In this case, however, given the very fast atmospheric diffusion rates, it would be difficult to account for the variable space distribution of 6-nitro-2,4-DCP across the delta water and, in particular, for the apparent dilution trend from the ditches into the lagoons. Finally, 6-nitro-2,4-DCP appeared to be somewhat more stable than 2,4-DCP since this chemical was

still detected on September 29th at a concentration level of 0.45 μg/L at the outlet of one ditch.

Field data would suggest a transformation process of 2,4-DCP into 6-nitro-2,4-DCP in the flooded fields or in the ditches/lagoons. Given the season when the 6-nitro-2,4-DCP buildup was observed, photonitration in surface water (30, 31) or a microbiological nitration process (32), although very little is known on the latter issue at the moment, might be reasonable tentative hypotheses. The following sections are an attempt to verify if the photochemical pathway is able to account for the field data.

Photoinduced Nitration Processes. First of all, it should be considered that 6-nitro-2,4-DCP might, in principle, arise from the nitration of 2,4-DCP or the dichlorination of 2-nitrophenol. As far as the second pathway is concerned, no formation of 6-nitro-2,4-DCP from 2-nitrophenol could be observed under conditions that can lead to aromatic chlorination (Fe(III) + Cl$^-$ + hν, TiO$_2$ + Cl$^-$ + hν, H$_2$O$_2$ + HCl) (26, 33). Accordingly, subsequent studies were focused on 2,4-DCP nitration.

The transformation of 2,4-DCP was studied under different conditions, among which nitrate photolysis and nitrite photooxidation by Fe(III) oxide colloids. Fe(III) oxides are photoactive semiconductors able to induce photochemical oxidation of a number of organic and inorganic compounds (31, 33, 34). The UV photolysis of nitrate yields reactive species such as $^\bullet$OH, $^\bullet$NO$_2$, and NO$_2^-$/HNO$_2$ (35), of which $^\bullet$NO$_2$ and HNO$_2$ are potential nitrating agents (36, 37). 2,4-DCP photonitration into 6-nitro-2,4-DCP under nitrate irradiation was strongly enhanced at acidic pH, coherently with a major role of HNO$_2$ under such conditions (37), which are, however, hardly relevant to surface waters.

Significant formation of 6-nitro-2,4-DCP at pH 7 could be detected on irradiation of 2,4-DCP in the presence of hematite (α-Fe$_2$O$_3$) or an equivalent amount of Fe$_2$(SO$_4$)$_3$ (forming Fe oxide colloids), and NaNO$_2$. The chosen pH conditions are representative of the values found in rice fields and ditches (6.8−7.2) and quite near to those in the lagoons (7.2−7.8). The yield of 6-nitro-2,4-DCP was around 38% of transformed 2,4-DCP. In comparison, note that the photonitration yield with nitrate at pH 6 was only 1%, most likely because nitrate photolysis produces $^\bullet$OH that involves 2,4-DCP into transformation pathways different from nitration. Coherently 2-hydroxy-3,5-dichloro-1,4-quinone, arising from multiple hydroxylation of the initial substrate, was the main detected transformation intermediate. The low nitration yield in the presence of substantial oxidation by $^\bullet$OH makes evidence against the importance of a possible nitration process initiated by $^\bullet$OH and involving $^\bullet$OH + $^\bullet$NO$_2$. Under field conditions, $^\bullet$OH would mainly be scavenged by dissolved organic matter (DOM) and not by 2,4-DCP, and would not decrease the nitration yield by $^\bullet$NO$_2$ significantly. Laboratory simulation of the role of DOM being not trivial, the Fe(III) + nitrite system would be very suitable to model the nitration of 2,4-DCP.

Photonitration of 2,4-DCP into 6-nitro-2,4-DCP by Fe-(III) oxide colloids and nitrite is most likely due to photoinduced nitrite oxidation to $^\bullet$NO$_2$, as already found in previous studies with phenol and nitrophenols (36, 38). Note that $^\bullet$-NO$_2$ photogeneration on nitrate photolysis or nitrite photooxidation is a potential source of nitroaromatic compounds in surface and atmospheric waters (39−41).

The assessment of the environmental significance of the photonitration of 2,4-DCP into 6-nitro-2,4-DCP requires the knowledge of the reaction kinetics between the substrate and $^\bullet$NO$_2$. A key prerequisite is the measurement of the steady-state [$^\bullet$NO$_2$] under the irradiation device adopted to study the photochemical generation of 6-nitro-2,4-DCP. It was, therefore, necessary to study a model reaction of known kinetics under the same conditions adopted for the photo-
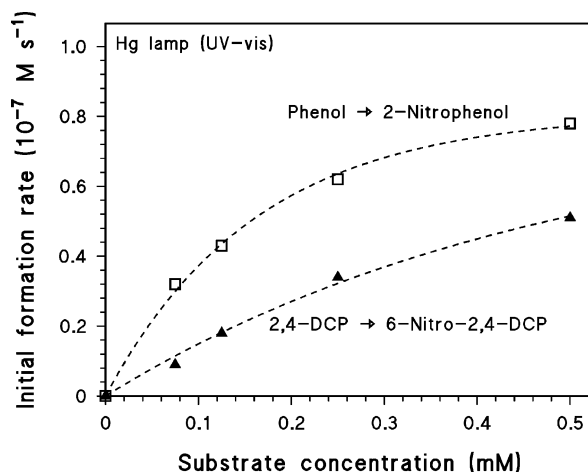

FIGURE 2. Initial formation rates of 2-nitrophenol and of 6-nitro-2,4-DCP as a function of the concentration of the substrates (phenol and 2,4-DCP, respectively). Other experimental conditions: α-Fe$_2$O$_3$ (100 mg L^{-1} Fe), 1.1 × 10^{-3} M NaNO$_2$, irradiation under medium-pressure mercury lamp (see the SI for the lamp emission spectrum).

production of 6-nitro-2,4-DCP. The transformation of phenol into 2-nitrophenol was chosen, described by the following kinetic equation (39):

$$d[\text{2-nitrophenol}]/dt = 1.92 \times 10^3 \, [\text{Phenol}] \, [^\bullet\text{NO}_2] \tag{1}$$

The steady-state [$^\bullet$NO$_2$] under given irradiation conditions is controlled by the formation−transformation budget. While the formation rate in the studied system (hematite + nitrite) would mainly be influenced by the photooxidation of nitrite, transformation would mainly depend on the hydrolysis of $^\bullet$NO$_2$ and the possible reaction between $^\bullet$NO$_2$ and the substrate. The latter reaction would be influenced by substrate concentration and, for low initial [substrate], hydrolysis is expected to prevail. The steady-state [$^\bullet$NO$_2$] would thus be substrate-dependent at high [substrate] and substrate-independent at low [substrate]. The data at low [substrate] are more significant because the information obtained with phenol nitration should be applied to the nitration of 2,4-DCP, and it is necessary that the steady-state [$^\bullet$NO$_2$] be not influenced by the nature and concentration of the substrate.

The initial formation rates of 2-nitrophenol from phenol and of 6-nitro-2,4-DCP from 2,4-DCP, in the presence of α-Fe$_2$O$_3$ (100 mg L^{-1} Fe) and 1.1 × 10^{-3} M NaNO$_2$ under irradiation, were studied as a function of [substrate] and are reported in Figure 2. The less than linear increase of the rates with [substrate] is motivated by the fact that, at sufficiently high [substrate], its reaction with $^\bullet$NO$_2$ starts to become important compared to $^\bullet$NO$_2$ hydrolysis. Because the application of eq 1 to the kinetics of the reaction 2,4-DCP + $^\bullet$NO$_2$ → 6-nitro-2,4-DCP requires a substrate-independent [$^\bullet$NO$_2$], the limit for [substrate] → 0 will be considered. Also note that the environmental concentration values of [2,4-DCP] are sufficiently low to make this compound a negligible sink for $^\bullet$NO$_2$. From the initial slope of the curve fitting the rate of 2-nitrophenol (Figure 2) one gets

$$\lim_{[\text{phenol}] \to 0} (d[\text{2} - \text{nitrophenol}]/dt) = 4.76 \times 10^{-4} \, [\text{phenol}] \tag{2}$$

The combination of eqs 1 and 2 yields [$^\bullet$NO$_2$] = 2.5 × 10^{-7} M under the adopted irradiation conditions. The [$^\bullet$NO$_2$] value thus obtained is substrate-independent; it can also be valid for the case of 2,4-DCP under the same irradiation conditions,

provided that the limit for zero substrate concentration is considered. Thus

$$\lim_{[2,4-DCP]\to 0} (d[6-nitro-2,4-DCP]/dt) = 1.6 \times 10^{-4}$$

$$[2,4-DCP] = k_3 \, [{}^\bullet NO_2][2,4-DCP] \quad (3)$$

From $[{}^\bullet NO_2] = 2.5 \times 10^{-7}$ M one gets $k_3 = 650$ M^{-1} s^{-1}.

Intermediates of 2,4-DCP Photodegradation. Various intermediates of 2,4-DCP photodegradation were detected by HPLC-MS^2 and identified on the basis of their mass fragmentation pattern. Byproduct molecular weight (MW) was assigned on the basis of the pseudo-molecular ion [M−H]$^-$. The byproduct structures were tentatively elucidated according to their MS^2 mass fragmentation pattern using the pseudo-molecular ion as precursor ion. Selective detection of nitroso and nitrated compounds was straightforward based on the observation of the diagnostic [M−H−NO]$^-$ product ion (42). The structure of the detected 2,4-DCP photodegradation intermediates is provided in Scheme 1 (SI). Additional data concerning mass spectra and total ion current chromatograms (TIC) are also reported as SI (Figures D, E).

Under UV irradiation in the presence of nitrite and Fe(III) colloids in solution at pH 7, compounds I, II, III, IV, V, plus an unidentified one (MW 203) were detected. Compound I (MW 191) has a MS^2 spectrum depicting a fragment ion at m/z 160 ([M−H−NO]$^-$) and was assigned the structure of a nitroso-derivative of 2,4-DCP. Compounds II (MW 207) and III (MW 207) should be two isomers. Compound III was identified as 6-nitro-2,4-DCP by comparing its MS^2 spectrum with that of an authentic standard. The structure of II (3-nitro-2,4-DCP) was assumed according to its MS^2 spectrum that was also characterized by a base peak at m/z 176 corresponding to NO losses. The MS^2 spectrum of IV (MW 223) showed a NO$_2$ loss (m/z 177) together with an OH loss (m/z 205). Chromatographic retention time of IV was shorter than those of II and III, and consequently, IV was tentatively identified as an hydroxy-2,4-dichloro-nitrophenol, although NO losses were not observed. Compound V (MW 192) might correspond to a byproduct with a high degree of oxidation due to its very short chromatographic retention time. The MS^2 spectrum of V revealed losses of 28 mass units, probably corresponding to CO losses and characteristic of quinone derivatives. In addition, a fragment ion at m/z 155 related to HCl losses might evidence the occurrence of chlorine atoms in the structure of V, which was assumed to be 2-hydroxy-3,5-dichloro-1,4-quinone. It is noteworthy that, among the phototransformation intermediates of 2,4-DCP, only III (6-nitro-2,4-DCP) could be monitored in the field because of standard availability.

Assessment of 2,4-DCP Photonitration by ${}^\bullet NO_2$ Under Field Conditions. Based on the transformation kinetics of 2,4-DCP into 6-nitro-2,4-DCP by ${}^\bullet NO_2$ (eq 3), it is possible to determine if the photonitration of 2,4-DCP can be compatible with field data, which requires an assessment of [${}^\bullet NO_2$] under field conditions.

Based on the results of previous studies, it is possible to approximately assess the steady-state [${}^\bullet OH$] and [${}^\bullet NO_2$] under 22 W m^{-2} sunlight UV irradiation given the water sample composition (39, 40). The method has been proven to foresee quite well the rate of phenol nitration on irradiation of natural water samples (39). The required analytical data were available for water in rice fields, ditches, and lagoons. Note that the main known photochemical ${}^\bullet NO_2$ sources in surface waters are nitrate photolysis and nitrite photooxidation by ${}^\bullet OH$ and irradiated Fe(III) compounds. On average, [NO$_3^-$] was 0.29 mM in rice fields, 0.15 in ditches, and 0.05 in lagoons, while the corresponding [NO$_2^-$] was 24, 12, and 3 μM, respectively (also see Table 1 in the SI). The role of Fe is the

most difficult to assess, and in this case it was assumed to vary from negligible to comparable with hematite as quantified in the present work (Figure 2 and eqs 1,2). Accordingly, the assessment of [${}^\bullet NO_2$] yielded an interval that depends on the hypothesized contribution of the Fe compounds (lower limit: no contribution; upper limit: contribution comparable to that of hematite). Details of the calculation procedure for [${}^\bullet OH$] and [${}^\bullet NO_2$] are given as SI.

Calculations yielded a steady-state [${}^\bullet NO_2$] (referred to 22 W m^{-2} sunlight UV intensity) of $1.5-2.4 \times 10^{-9}$ M in rice fields, $0.98-1.3 \times 10^{-9}$ M in ditches water, and $4.4-4.7 \times 10^{-10}$ M in lagoons. The differences are mainly accounted for by the decreasing concentration values of nitrate, nitrite and Fe in the three environments. From the calculated values of [${}^\bullet NO_2$] and eq 3 one gets the formation rate of 6-nitro-2,4-DCP for a given [2,4-DCP], and thus the time needed to reach a given [6-nitro-2,4-DCP] value.

The relevant calculations do, however, require a number of additional considerations. Quantitative field evidence of a possible transformation of 2,4-DCP into 6-nitro-2,4-DCP, accurate enough to enable calculations, was obtained in sampling site D1 (see Table 1 and Figure 1). The time interval between the observed concentration maxima of 2,4-DCP and 6-nitro-2,4-DCP was a couple of weeks (June 21st−July 5th). Given the observed transformation kinetics in the period June 21st−July 5th, 2,4-DCP had an average concentration of about 1.7×10^{-8} M. During the same period, 6-nitro-2,4-DCP showed an increase by about 5×10^{-9} M. However, it is noticeable that 6-nitro-2,4-DCP also underwent degradation in the field. In the absence of degradation processes, its concentration in the period under examination would have increased by around 8×10^{-9} M, obtained by extrapolation of the time evolution curve. Field data would suggest an overall nitration yield around 33% of the transformed substrate, which is compatible with the 6-nitro-2,4-DCP yield observed in the model photochemical system containing hematite + nitrite (38% of transformed 2,4-DCP), with ${}^\bullet NO_2$ as the nitrating agent.

Equation 3 with $k_3 = 650$ M^{-1} s^{-1} can be applied to the assessment of the transformation kinetics of 2,4-DCP into 6-nitro-2,4-DCP, with [2,4-DCP] = 1.7×10^{-8} M. The time scale of the process under 22 W m^{-2} sunlight UV would be the time required to reach 8×10^{-9} M 6-nitro-2,4-DCP, given the nitration rate from eq 3. However, transfer of constant (22 W m^{-2}) irradiation data to field conditions requires knowledge of the outdoor irradiation intensity. Measurement was carried out for 15 July at 45°N latitude (40), which will be adopted as a standard summer day. The amount of ${}^\bullet NO_2$ produced during the standard day would be equivalent to that generated on 10 h irradiation at 22 W m^{-2} sunlight UV (39). The time scale calculated on assumption of constant irradiation intensity can thus be formulated in terms of outdoor summer sunny days. t_{24N} is defined as the time that would be required to transform 1.7×10^{-8} M 2,4-DCP into 8×10^{-9} M 6-nitro-2,4-DCP under field conditions. The calculation procedure is as follows:

$$t_{24N}[\text{outdoor days}] = \frac{[6-nitro-2,4-DCP]}{36000 \cdot k_3 \cdot [2,4-DCP] \cdot [{}^\bullet NO_2]} \quad (4)$$

where [6-nitro-2,4-DCP] = 8×10^{-9} M, $k_3 = 650$ M^{-1} s^{-1}, [2,4-DCP] = 1.7×10^{-8} M, and [${}^\bullet NO_2$] depends on water composition. The value 36 000 s (outdoor days)$^{-1}$ is the conversion factor to take into account the equivalence between the summer sunny day taken as reference and the steady-state 22 W m^{-2} sunlight irradiation (10 h, ref 39).

The described procedure (also see the SI) yielded t_{24N} values of 8−13 days for rice field water, 15−21 for ditches, and 43−46 for lagoons. The t_{24N} interval derives from that of

[$^\bullet$NO$_2$], with the upper t_{24N} limit in case of no contribution by Fe to $^\bullet$NO$_2$ photoproduction, and the lower limit if hematite is representative of the average contribution of Fe species. In the latter case, the Fe contribution to $^\bullet$NO$_2$ would be highly significant. Additionally, nitrite photooxidation by $^\bullet$OH was more important than nitrate photolysis as $^\bullet$NO$_2$ source. Note that the reported t_{24N} values refer to the water surface layer, while photochemical reactions would be slower in whole water columns. However, when considering the absorption spectrum of delta water (*39*), water column effects should be very limited in the shallow layer of the rice fields (10 cm average depth) compared with ditches (50 cm) and lagoons (1 m). This is quite interesting because the evaluated time scale in the case of rice field water is compatible with the observed two weeks' transformation time of 2,4-DCP into 6-nitro-2,4-DCP. Note that the rice fields are also the place where herbicide application (dichlorprop) would primarily occur, and where the subsequent transformation into 2,4-DCP and the production of 6-nitro-2,4-DCP would be most likely.

Field data and the results of photochemistry studies are compatible with the occurrence of 6-nitro-2,4-DCP as the consequence of a photochemical nitration process of 2,4-DCP involving photogenerated $^\bullet$NO$_2$ in the shallow rice field water. Compatibility between field data and the hypothesized photochemical process includes both time scales and nitration yields (33−38%).

Acknowledgments

Supporting Information Available

Sampling site map, lamp emission spectrum, sample chromatograms, mass spectra of the monitored or otherwise identified compounds, structures of the 2,4-DCP phototransformation intermediates, and the calculation procedure for [$^\bullet$NO$_2$] in the field are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Palomo, M.; Bhandari, A. Time-dependent sorption-desorption behavior of 2,4-dichlorophenol and its polymerization products in surface soil. *Environ. Sci. Technol.* **2005**, *39*, 2143−2151.

(2) Palomo, M.; Bhandari, A. Impact of aging on the formation of bound residues after peroxidase-mediated treatment of 2,4-DCP contaminated soils. *Environ. Sci. Technol.* **2006**, *40*, 3402−3408.

(3) Crespin, M. A.; Gallego, M.; Valcarcel, M.; Gonzalez, J. L. Study of the degradation of the herbicides 2,4-D and MCPA at different depths in contaminated agricultural soil. *Environ. Sci. Technol.* **2001**, *35*, 4265−4270.

(4) Papaefthimiou, C.; Cabral, M. D.; Mixailidou, C.; Viegas, C. A.; Sa-Correia, I.; Theophilidis, G. Comparison of two screening bioassays, based on the frog sciatic nerve and yeast cells, for the assessment of herbicide toxicity. *Environ. Toxicol. Chem.* **2004**, *23*, 1211−1218.

(5) Zhang, H. C.; Huang, C. H. Oxidative transformation of triclosan and chlorophene by manganese oxides. *Environ. Sci. Technol.* **2003**, *37*, 2421−2430.

(6) Latch, D. E.; Pacher, J. L.; Stender, B. L.; VanOverbeke, J.; Arnold, W. A.; McNeill, K. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environ. Toxicol. Chem.* **2005**, *24*, 517−525.

(7) Yang, S.; Wu, R. S. S.; Kong, R. Y. C. Physiological and cytological responses of the marine diatom *Skeletonema costatum* to 2,4-dichlorophenol. *Aquat. Toxicol.* **2002**, *60*, 33−41.

(8) Scragg, A. H.; Spiller, L.; Morrison, J. The effect of 2,4-dichlorophenol on the microalga Chlorella VT-1. *Enzyme Microb. Technol.* **2003**, *32*, 616−622.

(9) Chen, J.; Jiang, J.; Zhang, F.; Yu, H.; Zhang, J. Cytotoxic effects of environmentally relevant chlorophenols on L929 cells and their mechanisms. *Cell Biol. Toxicol.* **2004**, *20*, 183−196.

(10) Bukowska, M. Effects of 2,4-D and its metabolite 2,4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. *Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol. Endocrinol.* **2003**, *135*, 435−441.

(11) Luo, Y.; Wang, X. R.; Shi, H. H.; Mao, D. Q.; Sui, Y. X.; Ji, L. L. Electron paramagnetic resonance investigation of in vivo free radical formation and oxidative stress induced by 2,4-dichlorophenol in the freshwater fish *Carassius auratus*. *Environ. Toxicol. Chem.* **2005**, *24*, 517−525.

(12) Sahinkaya, E.; Dilek, F. B. Effects of 2,4-dichlorophenol on activated sludge. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 361−367.

(13) Hale, D. D.; Rogers, J. E.; Wiegel, J. Environmental factors correlated to dichlorophenol dechlorination in anoxic freshwater sediments. *Environ. Toxicol. Chem.* **1991**, *10*, 1255−1265.

(14) Boule, P.; Guyon C.; Lemaire J. Photochimie et environnement VIII-Comportement photochimique de dichlorophenols en solution aqueuse diluée. *Chemosphere* **1984**, *5−6*, 603−612.

(15) Ukrainczyk, L.; McBride, M. B. Oxidation and dechlorination of chlorophenols in dilute aqueous suspensions of manganese oxides−Reaction products. *Environ. Toxicol. Chem.* **1993**, *12*, 2015−2022.

(16) Heng, Z. C.; Nath, J.; Liu, X. R.; Ong, T. Induction of chromosomal aberrations by 2,4-dichloro-6-aminophenol in cultured V79 cells. *Teratogen. Carcinogen. Mutagen.* **1996**, *16*, 81−87.

(17) Heng, Z. C.; Ong, T.; Nath, J. In vitro studies of the genotoxicity of 2,4-dichloro-6-nitrophenol ammonium (DCNPA) and its major metabolite. *Mutat. Res.* **1996**, *368*, 149−155.

(18) Kroegerkoepke, M. B.; Koepke, S. R.; Hernandez, L.; Michejda, C. J. Activation of a beta-hydroxyalkylnitrosamine to alkylating agents−Evidence for the involvement of a sulfotransferase. *Cancer Res.* **1992**, *52*, 3300−3305.

(19) Wang, L. Q.; James, M. O. Inhibition of sulfotransferases by xenobiotics. *Curr. Drug Metab.* **2006**, *7*, 83−104.

(20) Chiron, S.; Barbati, S.; De Méo, M.; Botta, A. In vitro synthesis of 1, N^6-etheno-2′-deoxyadenosine and 1, N^2-etheno-2′-deoxyguanosine by 2,4-dinitrophenol and 1,3-dinitropyrene in the presence of a bacterial nitroreductase. *Environ. Toxicol.*, in press.

(21) Cullen, J. T.; Bergquist, B. A.; Moffett, J. W. Thermodynamic characterization of the partitioning of iron between soluble and colloidal species in the Atlantic Ocean. *Mar. Chem.* **2006**, *98*, 295−303.

(22) Comoretto, L.; Arfib, B.; Chiron, S. Pesticides in the Rhône river delta: basic data for a field based risk assessment. *Sci. Total Environ.*, in press.

(23) Schüssler, W.; Nitschke, L. Nitrophenols in precipitation. *Chemosphere* **2001**, *42*, 277−283.

(24) Puig, D.; Silgoner, I.; Grasserbauer, M.; Barcelo, D. Part-per-trillion level determination of priority methyl-, nitro- and chlorophenols in river water samples by automated on-line liquid/solid extraction followed by liquid chromatography/mass spectrometry using atmospheric pressure chemical ionization and ion spray interfaces. *Anal. Chem.* **1997**, *69*, 2756−2761.

(25) Freitas, L.; Götz, C.; Ruff, M.; Singer, H.; Müller, S. Quantification of the new triketone herbicides, sulcotrione and mesotrione, and other important herbicides and metabolites, at ng/L level in surface waters using liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2004**, *1028*, 277−286.

(26) Vione, D.; Maurino, V.; Minero, C.; Calza, P.; Pelizzetti, E. Phenol chlorination and photochlorination in the presence of chloride ions in homogeneous aqueous solution. *Environ. Sci. Technol.* **2005**, *39*, 5066−5075.

(27) Reitzel, L.; Tuxen, N.; Ledin, A.; Bjerg, P. L. Can degradation products be used as documentation for natural attenuation of phenoxy acids in ground water? *Environ. Sci. Technol.* **2004**, *38*, 457−467.

(28) Bucheli, T.; Müller, S.; Heberle, S.; Schwarzenbach, R. Occurrence and behavior of pesticides in rainwater, roof runoff and artificial stormwater infiltration. *Environ. Sci. Technol.* **1998**, *32*, 3457−3464.

(29) Harrison, M. A. J.; Barra, S.; Borghesi, D.; Vione, D.; Arsene, C.; Olariu, R. I. Nitrated phenols in the atmosphere: A review. *Atmos. Environ.* **2005**, *39*, 231−248.

(30) Dzengel, J.; Theurich, J.; Bahnemann, D. W. Formation of nitroaromatic compounds in advanced oxidation processes: photolysis versus photocatalysis. *Environ. Sci. Technol.* **1999**, *33*, 294−300.

(31) Vione, D.; Maurino, V.; Minero, C.; Pelizzetti, E.; Harrison, M. A. J.; Olariu, R. I.; Arsene, C. Photochemical reactions in the

tropospheric aqueous phase and on particulate matter. *Chem. Soc. Rev.* **2006**, *35*, 441−453.

(32) Telscher, M. J. H.; Schuller, U.; Schmidt, B.; Schäffer, A. Occurrence of a nitro metabolite of a defined nonylphenol isomer in soil/sewage sludge mixtures. *Environ. Sci. Technol.* **2005**, *39*, 7896−7900.

(33) Chiron, S.; Minero, C.; Vione, D. Photodegradation processes of the antiepileptic drug carbamazepine, relevant to estuarine waters. *Environ. Sci. Technol.* **2006**, *40*, 5977−5983.

(34) Mazellier, P.; Sulzberger, B. Diuron degradation in irradiated, heterogeneous iron/oxalate systems: The rate-determining step. *Environ. Sci. Technol.* **2001**, *35*, 3314−3320.

(35) Mack, J.; Bolton, J. R. Photochemistry of nitrite and nitrate in aqueous solution: a review. *J. Photochem. Photobiol., A* **1999**, *128*, 1−13.

(36) Vione, D.; Maurino, V.; Minero, C.; Pelizzetti, E. Aqueous atmospheric chemistry: Formation of 2,4-dinitrophenol upon nitration of 2-nitrophenol and 4-nitrophenol in solution. *Environ. Sci. Technol.* **2005**, *39*, 7921−7931.

(37) Minero, C.; Bono, F.; Rubertelli, F.; Pavino, D.; Maurino, V.; Pelizzetti, E.; Vione, D. On the effect of pH in aromatic photonitration upon nitrate photolysis. *Chemosphere* **2007**, *66*, 650−656.

(38) Vione, D.; Maurino, V.; Minero, C.; Pelizzetti, E. New processes in the environmental chemistry of nitrite: Nitration of phenol upon nitrite photoinduced oxidation. *Environ. Sci. Technol.* **2002**, *36*, 669−676.

(39) Minero, C.; Chiron, S.; Falletti, G.; Maurino, V.; Pelizzetti, E.; Ajassa, R.; Carlotti, M.E.; Vione, D. Photochemical processes involving nitrite in surface water samples. *Aquat. Sci.* in press.

(40) Vione, D.; Falletti, G.; Maurino, V.; Minero, C.; Pelizzetti, E.; Malandrino, M.; Ajassa, R.; Olariu, R.-I.; Arsene, C. Sources and sinks of hydroxyl radicals upon irradiation of natural water samples. *Environ. Sci. Technol.* **2006**, *40*, 3775−3781.

(41) Minero, C.; Maurino, V.; Pelizzetti, E.; Vione, D. An empirical, quantitative approach to predict the reactivity of some substituted aromatic compounds towards reactive radical species ($Cl_2^{-\bullet}$, $Br_2^{-\bullet}$, $^{\bullet}NO_2$, $SO_3^{-\bullet}$, $SO_4^{-\bullet}$) in aqueous solution. *Environ. Sci. Pollut. Res.* **2006**, *13*, 212−214.

(42) Schmidt, A.; Herzschuh, R.; Matysik, F.; Engewald, W. Investigation of the ionisation behaviour of different nitroaromatic compounds occurring as polar metabolite of explosives using electrospray ionisation mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2293−2302.