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Laboratory and field evidence of the photonitration of 4-chlorophenol to 2-nitro-4-chlorophenol, and of the associated bicarbonate effect

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Abstract

Background, aim and scope. Photochemical processes can decontaminate the aqueous environment from xenobiotics, but they also produce secondary pollutants. This paper presents field and laboratory evidence of the transformation of 4-chlorophenol (4CP) into 2-nitro-4-chlorophenol (2N4CP).

Materials and methods. Field monitoring of 4CP and 2N4CP was carried out by solid phase extraction coupled with liquid chromatography-multiple reaction monitoring mass spectrometry. Laboratory irradiation experiments were carried out under a UV-Vis lamp, and the time evolution of the compounds of interest was followed by liquid chromatography.

Purpose. elucidating the pathways leading to 2N4CP from 4CP in paddy-field water.

Results and discussion. The field monitoring results suggest that 4CP can be transformed into 2N4CP in the paddy-field water of the Rhône delta (Southern France). The laboratory study indicates that the transformation can take place via photonitration by $^\bullet NO_2$. The nitration process is inhibited by bicarbonate, possibly due to basification that favours the occurrence of the 4-chlorophenolate. The latter could consume $^\bullet NO_2$ without being nitrated. The photonitration in the presence of bicarbonate could account for the observed transformation in the field.

Conclusions. Photonitration of 4CP to 2N4CP by $^\bullet NO_2$ could account for the observed interconversion of the two compounds in paddy fields. The results are of concern because 2N4CP is biorecalcitrant and toxic.

Recommendations and perspectives. Bicarbonate can modulate the photonitration of 4CP into 2N4CP, which can be very significant in bicarbonate-poor waters.

Keywords: Pesticide by-products, aromatic nitroderivatives, photochemistry, shallow surface waters.

1. Background, aim and scope

Pesticides are an important class of biorecalcitrant organic pollutants that can undergo a wide range of direct and indirect photochemical transformation reactions in surface waters (Lam et al. 2003). These processes influence considerably the environmental impact of pesticides, as far as decontamination issues and the generation of secondary pollutants are concerned. The flooded paddy fields can be very important compartments for the study of the photochemical processes, for their shallow water columns that favour the photochemical reactions and for the extensive use of pesticides in rice cultivation (Armbrust 1999). From this point of view the Rhône delta (Southern France) is an almost ideal study site because it is largely occupied by paddy fields, the water of which is rich of photoactive compounds (DOM, nitrate, nitrite, Fe) and is quite shallow (10 cm on average). The two combined effects can favour the photochemical processes to a significant extent (Chiron et al. 2007). Furthermore, the study of the impact of the pesticides and their transformation intermediates on the delta waters (Comoretto et al. 2007) is important because the area is one of the main wintering sites for waterbirds in the Mediterranean region, including large colonies of European flamingos (*Phoenicopterus roseus*).

4-Chlorophenol is a toxic compound, which targets cell mitochondria by acting as an uncoupling agent in oxidative phosphorylation (Narasimhan et al. 1992). It can be directly emitted to surface waters, but it is also derived from the transformation of the herbicide 2-(2,4-dichlorophenoxy)propanoic acid (dichlorprop (DCP); Comoretto et al. 2007). This paper reports on the photonitration of 4-chlorophenol (4CP) into 2-nitro-4-chlorophenol (2N4CP). It includes both field data about the possible interconversion of the two compounds in the paddy-field water of the Rhône delta, and a laboratory study of the photonitration process, its kinetics, and the associated effect of bicarbonate. It has recently been shown that the bicarbonate anions can considerably affect aromatic photonitration processes. They modify the pH of the solution, consume the $^\bullet OH$ radicals produced upon irradiation of nitrate and nitrite, and enhance the generation of the radicals $CO_3^{-\bullet}$ (Chiron et al. 2009a). The overall effect is strongly substrate-dependent (Vione et al. 2009). The formation of 2N4CP as a secondary pollutant is of concern because this compound is refractory to biological degradation, and it is toxic to organisms (The Merck Index 2006).
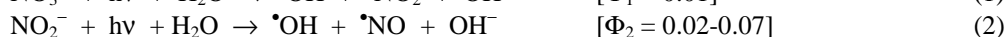
2. Materials and methods

The adopted reagents are listed in the Electronic Supplementary Material. Surface water was sampled from the Rhône delta (for a map of the sampling sites see Figure A in the Electronic Supplementary Material), transported under refrigeration to the laboratory, and filtered on 0.45 µm filter membranes. Sample pH was adjusted to a value of 5 with sulfuric acid (H_2SO_4), 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 liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), using an atmospheric pressure chemical ionisation (APCI) interface in negative ionisation mode, as recommended for chloro- and nitrophenols (Puig et al. 1997). 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 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 then back to the initial conditions in 5 min. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode with unit mass resolution. Deuterated 2,4-dichlorophenoxyacetic acid (2,4-D d_3) was added as isotope labelled internal standard. The selected transition ions were 127>127, 172>142, 233>161 and 222>164 for 4CP, 2N4CP, DCP and 2,4-D d_3, respectively. The percent recovery was 68±11% and 86±7% for 4CP and 2N4CP, respectively. The respective limits of quantification (LOQs) were 0.04 and 0.02 nM. Quantification was carried out by means of an isotope labelled 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 (Zhao and Metcalfe 2008). 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 were carried out under a Philips TL 01 UV-Vis lamp, with maximum emission at 313 nm and a UV irradiance (290-400 nm) of 5.6 W m^{-2}, measured with a CO.FO.ME.GRA. (Milan, Italy) power meter and corresponding to a volumetric photon flux of 3.7×10^{-6} einstein L^{-1} s^{-1} in solution. Figure 1 reports the emission spectrum of the lamp, measured with an Ocean Optics SD2000 CCD spectrophotometer, and the absorption spectrum of nitrate, measured with a Varian Cary 100 Scan UV-Vis spectrophotometer. The solutions (5 mL) were placed into Pyrex glass cells (4.0 cm diameter, 2.3 cm height), and irradiated under magnetic stirring. After irradiation, the time evolution of 4CP and of 2N4CP was monitored by High Performance Liquid Chromatography coupled with UV detection (HPLC-UV). The Merck-Hitachi instrument was equipped with AS2000A autosampler (standard sample volume was 100 µL), L-6200 and L-6000 pumps for high-pressure gradients, Merck LiChrocart RP-C18 column packed with LiChrospher 100 RP-18 (125 mm × 4.6 mm × 5 µm), and L-4200 UV-Vis detector (detection wavelength 210 nm). Samples were eluted with a 40/60 mixture of acetonitrile/aqueous H_3PO_4 (pH 2.8), at 1.0 mL min^{-1} flow rate. Retention times were (min): 4CP (3.6), 2N4CP (6.8), column dead time 0.9 min. The time evolution of 2N4CP in the laboratory experiments was fitted with equations like $[2N4CP]_t = k^f_{2N4CP} [4CP]_0 (k^d_{2N4CP} - k^d_{4CP})^{-1} [exp(-k^d_{4CP} t) - exp(-k^d_{2N4CP} t)]$, where $[2N4CP]_t$ is the concentration at the time t, $[4CP]_0$ the initial concentration of 4CP, k^f_{2N4CP} the pseudo-first order rate constant for the formation of 2N4CP, k^d_{4CP} and k^d_{2N4CP} the pseudo-first order rate constants for the degradation of 4CP and of 2N4CP, respectively. Note that it is $k^f_{2N4CP} < k^d_{4CP}$ because 2N4CP is not the only transformation intermediate of 4CP. The initial formation rate of 2N4CP (t = 0) would be $R_{2N4CP} = k^f_{2N4CP} [4CP]_0$. The error bounds to the rate data represent µ±σ, calculated from the scattering of the experimental data in a single run (intra-series variability). The reproducibility of repeated runs (inter-series variability) was in the range 10-15%.

3. Results and Discussion

Field data. Figure 2 reports the time evolution of 4CP and of 2N4CP in one of the sampling sites of the Rhône delta, a ditch that drains the paddy-field water into the Rhône river. One can note the increase of 4CP during the month of May, the peak in June, and the peak of 2N4CP two weeks later. Due to sampling frequency issues, the time interval between the two maxima as obtained from the monitoring study could be an approximation of the actual one. The reported trend was also observed in other sampling sites (see Table 1). 4CP is a possible intermediate of the direct photolysis of DCP (Climent and Miranda 1997; Meunier et al. 2002), a post-emergent herbicide used for rice crops in the delta (Comoretto et al. 2007). The time evolution of DCP is also reported in Figure 2 and in Table 1. Note that no DCP, 4CP or 2N4CP could be detected in rain water samples, thus excluding wet deposition of these compounds into the delta waters. Direct photolysis, reaction with $^\bullet OH$ and other transient species, and nitration are possible transformation pathways in paddy-field water (Armbrust 1999; Chiron et al. 2009). Nitroderivatives like 2N4CP absorb a larger fraction of sunlight compared with 4CP, but they have lower quantum yields for the direct photolysis (Vione et al. 2009). Additionally, the nitro group is expected to decrease the reaction rate constants with $^\bullet OH$ and other transient species (Neta et al. 1988; Minero et al. 2006), and would inhibit further nitration processes (Machado and Boule 1995; Dzengel et al. 1999).

Irradiation experiments. The field data could be compatible with a nitration process that transforms 4CP into 2N4CP. For this reason, a laboratory study was carried out of the possible processes that can lead to 2N4CP from 4CP. It was excluded a significant formation of 2N4CP upon chlorination of 2-nitrophenol, under conditions that lead for instance to the efficient chlorination of phenol ($FeCl_3$ or α–Fe_2O_3 + Cl^- under irradiation; Khanra et al. 2008). The nitration of 4CP into 2N4CP was observed in the presence of 4CP + NO_3^- + hv, 4CP + NO_2^- + hv, 4CP + NO_3^- + NO_2^- + hv, and 4CP + HNO_2 in the dark. A rate constant of 5.8×10^{-3} M^{-1} s^{-1} was measured for the reaction 4CP + $HNO_2 \rightarrow$ 2N4CP, but significant formation of 2N4CP only takes place at pH 5.5 or lower. The water pH in the Rhône delta (river, paddy fields, ditches and lagoons) is between 6.8 and 8.3, which excludes a significant role of thermal nitration by HNO_2.

The nitration of aromatic compounds by nitrate, nitrite, and nitrate + nitrite upon irradiation under circumneutral conditions is accounted for by photogenerated $^\bullet NO_2$ (Machado and Boule 1995; Dzengel et al. 1999; Mack and Bolton 1999; Minero et al. 2007a):

$$NO_3^- + hv + H_2O \rightarrow {}^\bullet OH + {}^\bullet NO_2 + OH^- \qquad [\Phi_1 = 0.01] \qquad\qquad (1)$$
$$NO_2^- + hv + H_2O \rightarrow {}^\bullet OH + {}^\bullet NO + OH^- \qquad [\Phi_2 = 0.02\text{-}0.07] \qquad (2)$$
$$NO_2^- + {}^\bullet OH \rightarrow {}^\bullet NO_2 + OH^- \qquad\qquad [k_3 = 1.0 \times 10^{10}\ M^{-1}\ s^{-1}] \qquad (3)$$

The formation rate of 2N4CP was significantly higher with 4CP + NO_3^- + NO_2^- + hv compared with 4CP + NO_3^- + hv. The photolysis of nitrate alone produces $^\bullet NO_2$ in reaction (1), together with $^\bullet OH$. If nitrite is also present in the system, further $^\bullet NO_2$ is generated in reaction (3) between nitrite and $^\bullet OH$. Moreover, the addition of 2-propanol as $^\bullet OH$ scavenger inhibited the generation of 2N4CP from 4CP + NO_2^- + hv. The photoproduction of $^\bullet NO_2$ by nitrite involves reactions (2,3), and the scavenging of $^\bullet OH$ by 2-propanol prevents the formation of $^\bullet NO_2$ in reaction (3).

It is possible to measure the photonitration rate constants of aromatic compounds by comparison with phenol (Chiron et al. 2007). The photonitration of phenol by $^\bullet NO_2$ yields both 2-nitrophenol and 4-nitrophenol (hereafter their sum will be indicated as NP). Let consider the nitration of phenol and 4CP by $^\bullet NO_2$ (reactions 4 and 5, respectively):

$$Phenol + {}^\bullet NO_2 \rightarrow NP \qquad\qquad [k_4 = 3.2 \times 10^3\ M^{-1}\ s^{-1}] \qquad (4)$$
$$4CP + {}^\bullet NO_2 \rightarrow 2N4CP \qquad\qquad [k_5] \qquad\qquad\qquad (5)$$

From reactions (4,5) one gets: $d[NP]/dt = k_4\ [Phenol]\ [^\bullet NO_2]$ and $d[2N4CP]/dt = k_5\ [4CP]\ [^\bullet NO_2]$. If one studies the two photonitration processes separately but under comparable conditions (*e.g.* with 0.10 M NO_3^-, under the same irradiation device), the generation rate of $^\bullet NO_2$ in reaction (1) is expected to be the same in both cases. Note that phenol and 4CP do not compete with nitrate for radiation absorption at 313 nm. The transformation of $^\bullet NO_2$ can take place via dimerisation followed by hydrolysis (reactions (6,7); Fischer and Warneck, 1996), or upon reaction with the aromatic substrate (phenol or 4CP depending on the system under study). It is reasonable that reactions (6,7) prevail as sinks of $^\bullet NO_2$ at low substrate concentration, and play a secondary role at elevated substrate concentration. If reactions (6,7) prevail as $^\bullet NO_2$ sinks, they would have the same rate with both phenol and 4CP. Therefore, under conditions of equal generation rate of nitrogen dioxide, the steady-state $[^\bullet NO_2]$ is expected to be the same with both compounds.

$$2\ {}^\bullet NO_2 \rightleftarrows N_2O_4 \qquad\quad [k_6 = 4.5 \times 10^8\ M^{-1}\ s^{-1};\ k_{-6} = 6.9 \times 10^3\ s^{-1}] \qquad (6)$$
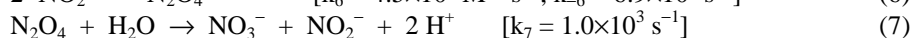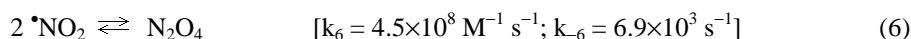$$N_2O_4 + H_2O \rightarrow NO_3^- + NO_2^- + 2\ H^+ \qquad [k_7 = 1.0 \times 10^3\ s^{-1}] \qquad (7)$$

Figure 3 reports the initial formation rates of NP from phenol and of 2N4CP from 4CP, in the presence of 0.10 M $NaNO_3$ under irradiation, as a function of the initial substrate concentration. For comparison they are also reported the corresponding data for the photonitration of *ortho*-cresol (2-methylphenol), which is a major by-product of 4-chloro-2-methylphenol, in turn a transformation intermediate of the herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA; Chiron et al. 2009b). Note the elevated formation rate of 2N4CP from 4CP, compared to the photonitration of phenol or cresol. At [Substrate] $\rightarrow$ 0, $[^\bullet NO_2]$ would be the same for both phenol and 4CP under nitrate irradiation. By substituting equal $[^\bullet NO_2]$ into the expressions of d[NP]/dt and of d[2N4CP]/dt, one gets k_5 as follows:

$$k_5 = k_4 \cdot \lim_{\substack{[Phenol] \to 0 \\ [4CP] \to 0}} \left(\frac{d[2N4CP]/dt}{[4CP]} \cdot \frac{[Phenol]}{d[NP]/dt} \right) \qquad (8)$$

where $k_4 = 3.2 \times 10^3$ M^{-1} s^{-1} (Chiron et al. 2007). By application of equation (8) to the experimental data of Figure 3 one gets $k_5 = 1.1 \times 10^4$ M^{-1} s^{-1}. The value of k_5 is referred to the undissociated 4CP, because this compound has $pK_a \approx 9.4$

(Martell et al., 1997) and the irradiation experiments were carried out at pH around 6. The value of k_5 is very important because it enables the comparison of laboratory and field data. In the paddy-field water of the Rhône delta one can estimate that $[{}^\bullet NO_2] = (1.5 \div 2.4) \times 10^{-9}$ M under 22 W m^{-2} sunlight UV irradiance (Chiron et al. 2007). The field data (Figure 2) suggest that in the two weeks from 21 June to 5 July, $[4CP] \approx 1.2 \times 10^{-9}$ M on average. In the same period the concentration of 2N4CP increased up to a maximum. The shape of the curve indicates that 2N4CP was also degraded, thus the observed 5×10^{-10} M increase from 21 June to 5 July would probably make 6×10^{-10} M without degradation. Also note that in a whole summer sunny day the sunlight energy reaching the ground would be equivalent to about 10 hour (3.6×10^4 s) continuous illumination at 22 W m^{-2} UV irradiance (Minero et al. 2007b). The time (in sunny day units) required to transform 1.2×10^{-9} M 4CP into 6×10^{-10} M 2N4CP in the water of the paddy fields is:

$$t_{2N4CP}[sunny\ days] = \frac{[2N4CP]}{3.6 \cdot 10^4 \cdot k_5 \cdot [4CP] \cdot [{}^\bullet NO_2]} \qquad (9)$$

$[{}^\bullet NO_2] = (1.5 \div 2.4) \times 10^{-9}$ M is referred to 22 W m^{-2} sunlight UV irradiance. By substituting the values one obtains t_{2N4CP} = 0.53÷0.84 sunny days. Even by considering that the transformation time derived from the field monitoring (2 weeks) is just an approximation because of a sampling frequency bias, there is a clear disagreement between the field data and the calculation results. However, it should be considered that the water of the paddy fields also contains 13 mg C L^{-1} Non-Purgeable Organic Carbon (NPOC), and 3.1 mM bicarbonate. The content of organic carbon would not be high enough for the dissolved organic matter to scavenge ${}^\bullet NO_2$ faster than reactions (6,7) (Chiron et al. 2007). However, bicarbonate and carbonate have been shown to affect the photonitration reactions to a very significant extent (Chiron et al. 2009a). For this reason, it was studied the effect of bicarbonate on the formation of 2N4CP from 4CP.

Effect of bicarbonate on the photonitration of 4CP. Figure 4 reports the initial formation rate of 2N4CP from 1 mM 4CP, upon irradiation of 0.10 M nitrate, as a function of the concentration of NaHCO$_3$. The formation of 2N4CP is considerably inhibited by bicarbonate, and levels off above 0.2 mM NaHCO$_3$. The latter effect is parallel to the stabilisation of pH at around 8.2 with increasing NaHCO$_3$.
Bicarbonate above 5 mM has been shown to enhance the photonitration of phenol upon nitrate irradiation. Such an effect, which is independent of pH in the basic range, could be attributed to the reaction between photogenerated peroxynitrite and bicarbonate to yield additional ${}^\bullet NO_2$ (reactions (10,11); Chiron et al. 2009a).

$$NO_3^- + h\nu \rightarrow ONOO^- \qquad (10)$$
$$ONOO^- + HCO_3^- \rightarrow {}^\bullet NO_2 + CO_3^{-\bullet} + OH^- \qquad (11)$$

Reactions (10,11) are probably operational also with 4CP, but in this case bicarbonate inhibits photonitration. The addition of NaOH also inhibited the photonitration of 4CP by nitrate, thus the effect of bicarbonate is probably linked to pH. Moreover, no photonitration of 4-chlorophenolate (4CP$^-$) was observed upon irradiation of nitrate above pH 10. A possible explanation for the pH effect could be a competition between 4CP and 4CP$^-$ for reaction with ${}^\bullet NO_2$. Phenolate and the methylphenolates react with ${}^\bullet NO_2$ by one-electron oxidation, with rate constants of about 10^7 M^{-1} s^{-1} (Neta et al. 1988; Minero et al. 2006). The rate constant of 4CP$^-$ is not available, but if it were of the same order of magnitude, a small percentage of 4CP$^-$ would be sufficient to scavenge most ${}^\bullet NO_2$ at pH 8-8.2 (4CP has pK$_a$ ~ 9.4). The pH increase upon addition of NaHCO$_3$ would favour the occurrence of 4CP$^-$, which might effectively compete with 4CP for ${}^\bullet NO_2$. Less ${}^\bullet NO_2$ would thus be available for the nitration of 4CP, while the reaction between the 4CP$^-$ and ${}^\bullet NO_2$ would not yield 2N4CP significantly. The overall result would be an inhibition of 4CP nitration by bicarbonate. The ineffective photonitration of 4CP$^-$ is not a unique case. For instance, the nitration of phenolate by ${}^\bullet NO_2$ to yield 2-nitrophenolate is considerably less efficient than the corresponding nitration of the undissociated phenol (Vione et al. 2004).
In the presence of 3 mM HCO$_3^-$ (a similar concentration and similar pH as that of the paddy fields of the Rhône delta), the formation rate of 2N4CP is around 17 times lower than in the absence of bicarbonate. Therefore, t_{2N4CP} would be proportionally increased to 9-14 days, which is compatible with field data. It is possible to conclude that the field data of 4CP and 2N4CP in the Rhône delta water are compatible with a photonitration process that transforms the former into the latter, and which is modulated by the presence of dissolved bicarbonate.

4. Conclusions

Field monitoring results referred to the paddy fields of the Rhône delta suggest a possible nitration pathway of 4CP into 2N4CP. The nitration rate constant of the undissociated 4CP into 2N4CP is 1.1×10^4 M^{-1} s^{-1}. It is around 3.5 times

higher than the nitration rate constant of phenol into 2- and 4-nitrophenol, and four times higher than the rate constant for the nitration of cresol. Therefore, 4CP undergoes photonitration rather easily. However, the field data do not match such a fast nitration kinetics: the laboratory results suggest a time scale of less than one day for the transformation of 4CP into 2N4CP, to be compared with the two weeks' time for nitration in the field. The most likely explanation for such a difference is that the photonitration of 4CP into 2N4CP is significantly inhibited by bicarbonate. The addition of $NaHCO_3$ increases both the solution pH and the ratio of $4CP^-$ to 4CP. If the reaction rate constant between $4CP^-$ and $^\bullet NO_2$ was around 10^7 M^{-1} s^{-1}, like those of other phenolates, $4CP^-$ would scavenge the majority of photogenerated $^\bullet NO_2$ at pH 8-8.2. Nitrogen dioxide would therefore be subtracted to the nitration of 4CP, and its reaction with $4CP^-$ would yield insignificant nitration. When considering the inhibition effect carried out by bicarbonate, a reasonable match between the predictions based on laboratory data and the field results could be obtained. Bicarbonate is able to modulate the photonitration of 4CP. The field data suggest that the formation of 2N4CP is an important transformation pathway for 4CP, and it could be even more significant in bicarbonate-poor waters.

5. Recommendations and Perspectives

This work shows the potential for the photochemical formation of toxic and potentially mutagenic (Heng et al. 1996) aromatic nitroderivatives in paddy fields. 4CP could be an important compound in this respect because of its fast photonitration kinetics, most notably in bicarbonate-poor waters. Previous studies have shown that bicarbonate can have a major influence on photonitration processes and on the transformation of aromatic compounds upon nitrate photolysis (Chiron et al. 2009a; Vione et al. 2009). This is the first work to our knowledge that provides field data to demonstrate the importance of the bicarbonate effect on photonitration. Several studies about the paddy fields of the Rhône delta suggest that efficient nitration of pesticide by-products (2,4-dichlorophenol, 4-chloro-2-methylphenol, 4-chlorophenol) can take place in this environment (Chiron et al. 2007; Chiron et al. 2009b; this work). It is therefore recommended that chlorophenols and their nitroderivatives be included in field monitoring programmes intended to assess the environmental occurrence and impact of pesticides such as dichlorprop and MCPA.

References

Armbrust KL (1999) Photochemical processes influencing pesticide degradation in rice paddies. J Pesticide Sci 24:69-73

Chiron S, Minero C, Vione D (2007) Occurrence of 2,4-dichlorophenol and of 2,4-dichloro-6-nitrophenol in the Rhône river delta (Southern France). Environ Sci Technol 41:3127-3133

Chiron S, Barbati S, Khanra S, Dutta BK, Minella M, Minero C, Maurino V, Pelizzetti E, Vione D (2009a) Bicarbonate-enhanced transformation of phenol upon irradiation of hematite, nitrate, and nitrite. Photochem Photobiol Sci 8:91-100

Chiron S, Comoretto L, Rinaldi E, Maurino V, Minero C, Vione D (2009b) Pesticide by-products in the Rhône delta (Southern France). The case of 4-chloro-2-methylphenol and of its nitroderivative. Chemosphere 74:599-604

Climent MJ, Miranda MA (1997) Photodegradation of dichlorprop and 2-naphtoxyacetic acid in water. Conmbined GC-MS and GC-FTIR study. J Agric Food Chem 45:1916-1919

Comoretto L, Arfib B, Chiron S (2007) Pesticides in the Rhône river delta (France): basic data for a field-based exposure assessment. Sci Total Environ 380:124-132

Dzengel J, Theurich J, Bahnemann DW (1999) Formation of nitroaromatic compounds in advanced oxidation processes: photolysis versus photocatalysis. Environ Sci Technol 33:294-300

Fischer M, Warneck P (1996) Photodecomposition of nitrite and undissociated nitrous acid in aqueous solution. J Phys Chem 100:18749-18756

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

Khanra S, Minero C, Maurino V, Pelizzetti E, Dutta BK, Vione D (2008) Phenol transformation induced by UVA photolysis of the complex $FeCl^{2+}$. Environ Chem Lett 6:29-34

Lam MW, Tantuco K, Mabury SA (2003) PhotoFate: a new approach in accounting for the contribution of indirect photolysis of pesticides and pharmaceuticals in surface waters. Environ Sci Technol 37:899-907

Machado F, Boule P (1995) Photonitration and photonitrosation of phenolic derivatives induced in aqueous solutions by excitation of nitrite and nitrate ions. J Photochem Photobiol A: Chem 86:73-80

Mack J, Bolton JR (1999) Photochemistry of nitrite and nitrate in aqueous solution: a review. J Photochem Photobiol A: Chem 128:1-13

Martell AE, Smith RM, Motekaitis RJ (1997) Critically selected stability constants of metal complexes database, version 4.0, released November 1997 (NIST)

Meunier L, Gauvin E, Boule P (2002) Photochemical behaviour of dichlorprop [(+/-)-2-(2,4-dichlorophenoxy)propanoic acid] in aqeuous solution. Pest Manag Sci 58:845-852

Minero C, Maurino V, Pelizzetti E, Vione D (2006) 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 13:212-214

Minero C, Maurino V Pelizzetti E, Vione D (2007a) Assessing the steady-state [$\bullet$NO$_2$] in environmental samples. Implications for aromatic photonitration processes induced by nitrate and nitrite. Environ Sci Pollut Res 14:241-243.

Minero C, Chiron S, Falletti G, Maurino V, Pelizzetti E, Ajassa R, Carlotti ME, Vione D (2007b) Photochemical processes involving nitrite in surface water samples. Aquat Sci 69:71-85

Narasimhan TR, Mayura K, Clement BA, Safe SH, Phillips TD (1992) Effects of chlorinated phenols on rat embryonic and hepatic mitochondrial oxidative-phosphorylation. Environ Toxicol Chem 11:805-814

Neta P, Huie RE, Ross AB (1988) Rate constants for reactions of inorganic radicals in aqueous solution. J Phys Chem Ref Data 17:1027-1229

Puig D, Silgoner I, Grasserbauer M, Barcelo D (1997) 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 69:2756-2761

The Merck Index (2006) An Encyclopaedia of Chemicals, Drugs and Biologicals. Merck Research Laboratories, Whitehouse Station, NJ, USA

Vione D, Maurino V, Pelizzetti E, Minero C (2004) Phenol photonitration and photonitrosation upon nitrite photolysis in basic solution. Intern J Environ Anal Chem 84:493-504

Vione D, Khanra S, Cucu Man S, Maddigapu PR, Das R, Arsene C, Olariu RI, Maurino V, Minero C (2009) Inhibition vs. enhancement of the nitrate-induced phototransformation of organic substrates by the $\bullet$OH scavengers bicarbonate and carbonate. Wat Res 43:4718-4728

Zhao X, Metcalfe C (2008) Characterizing and compensating for matrix effects using atmospheric pressure chemical ionization liquid chromatography mass spectrometry: Analysis of neutral pharmaceuticals in municipal wastewater. Anal Chem 80:2010-2017.

Table 1. Analytical data of DCP, 4CP and 2N4CP in the different sampling sites and dates. D1, D2: ditches, L1, L2: lagoons, S1: sampling station at the inlet of the delta. For a map of the sampling sites see Figure A in the Electronic Supplementary Material. Error bounds: ±1 standard deviation of triplicate runs.

| Compound	Sampling date	D1	D2	L1	L2	S1
DCP (10^{-9} **M**)	19Apr05	n.d	n.d	n.d	n.d	n.d
	20May05	0.17±0.03	n.d	n.d	n.d	n.d
	21Jun05	13.2±1.6	3.0±0.4	0.47±0.04	0.34±0.04	n.d
	5Jul05	1.7±0.2	0.77±0.09	0.26±0.03	n.d	n.d
	18Jul05	n.d	0.21±0.03	n.d	n.d	n.d
	24Aug05	n.d	n.d	n.d	n.d	n.d
	22Sep05	n.d	n.d	n.d	n.d	n.d
4CP (10^{-9} **M**)	19Apr05	n.d	n.d	n.d	n.d	n.d
	20May05	0.05±0.01	n.d	n.d	n.d	n.d
	21Jun05	1.71±0.18	0.43±0.05	0.19±0.03	0.21±0.03	n.d
	5Jul05	0.58±0.08	0.28±0.03	0.08±0.01	0.11±0.01	n.d
	18Jul05	0.37±0.05	0.09±0.01	n.d	n.d	n.d
	24Aug05	n.d	n.d.	n.d	n.d	n.d
	22Sep05	n.d	n.d.	n.d	n.d	n.d
2N4CP (10^{-9} **M**)	19Apr05	n.d.	n.d.	n.d.	n.d.	n.d
	20May05	n.d	n.d	n.d.	n.d	n.d
	21Jun05	0.22±0.02	0.08±0.01	n.d	n.d	n.d
	5Jul05	0.65±0.07	0.25±0.03	0.08±0.01	0.12±0.02	n.d
	18Jul05	0.41±0.05	0.06±0.01	0.05±0.01	0.08±0.01	n.d
	24Aug05	n.d	n.d	n.d	n.d	n.d
	22Sep05	n.d	n.d	n.d	n.d	n.d

n.d: not determined (below detection limit)
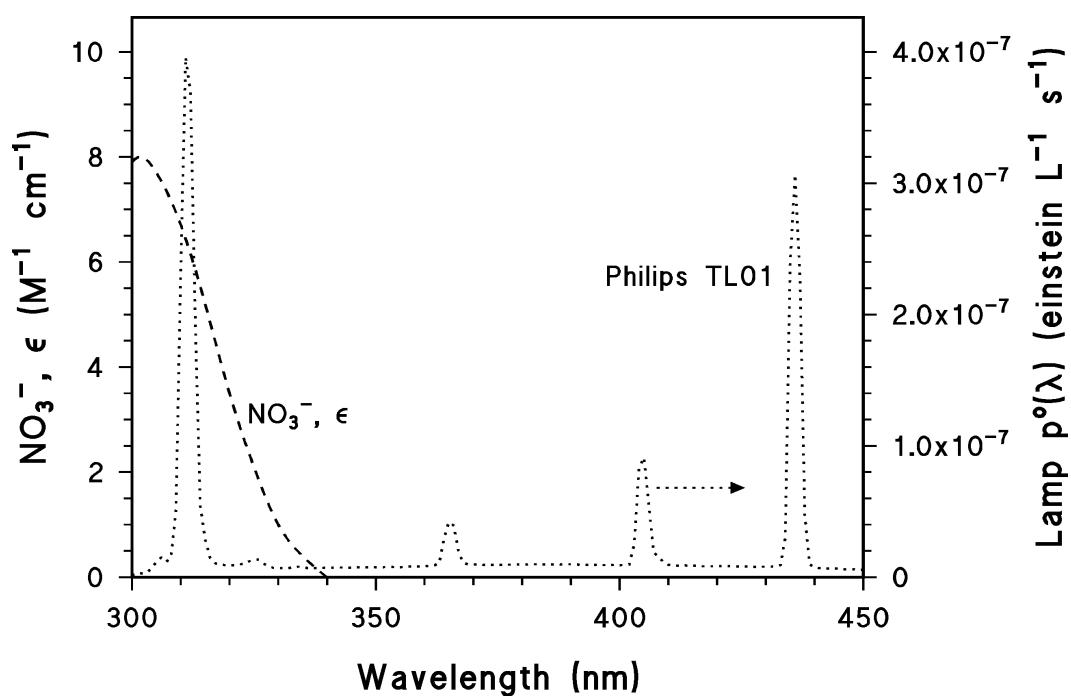
Figure 1. Emission spectrum of the adopted UV-Vis lamp. Absorption spectrum of nitrate (molar absorption coefficient, M^{-1} cm^{-1}).
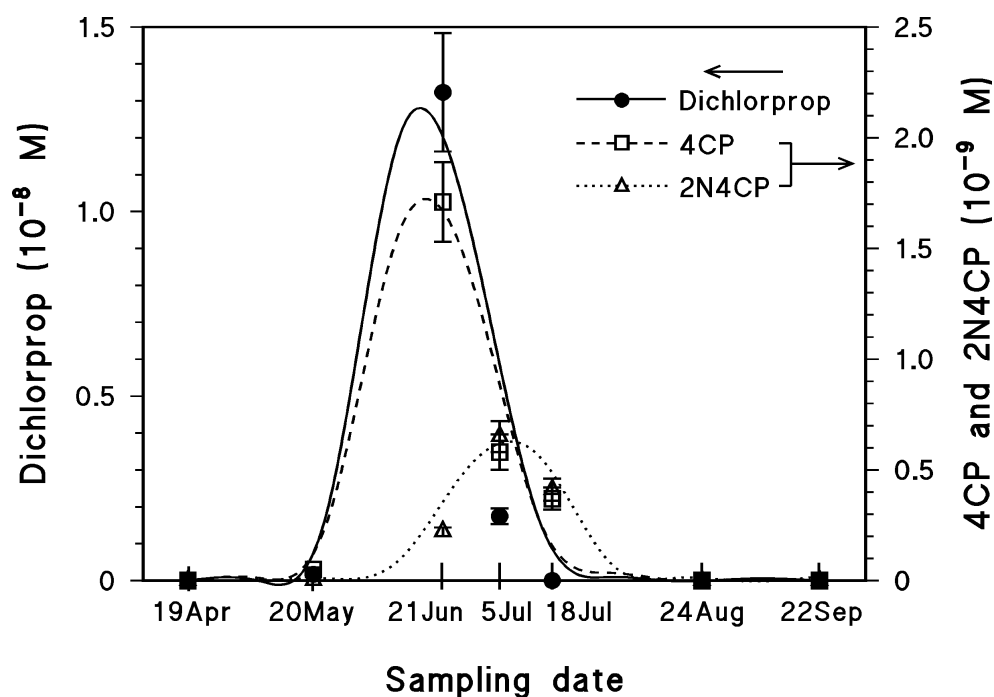

Figure 2. Time evolution of DCP, 4CP and 2N4CP in sampling site D1 (ditch, which drains the paddy field water into the Rhône river) during the year 2005. Note the two weeks' separation time between the concentration maxima of DCP and 4CP on the one hand, and that of 2N4CP on the other.
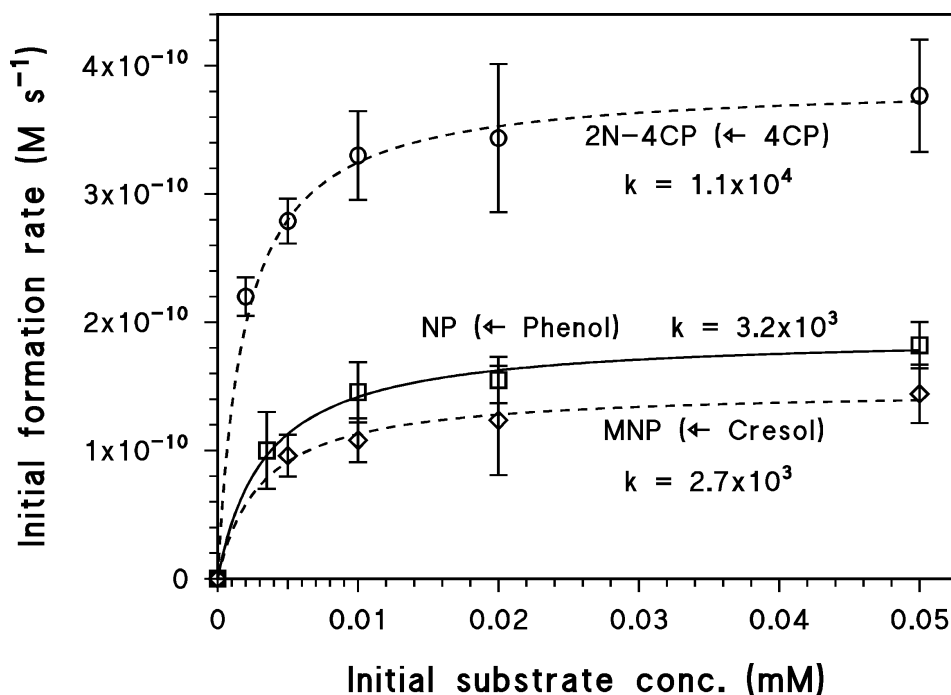
Figure 3. Initial formation rates of 2- and 4-nitrophenol (NP) from phenol, of 2N4CP from 4CP, and of 4-nitro-2-methylphenol (MNP) from 2-methylphenol (Cresol), as a function of the concentration of the aromatic substrate (Phenol, 4CP, or Cresol). Initial nitrate concentration was 0.10 M, irradiation was carried out under the Philips TL 01 lamp.
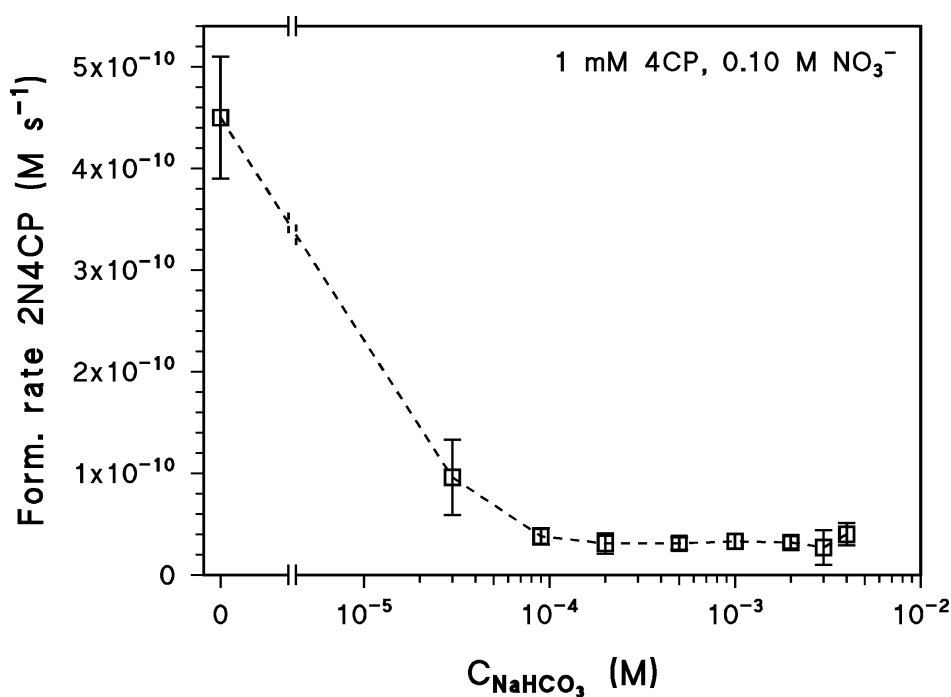
Figure 4. Initial formation rates of 2N4CP from 1 mM 4CP, upon irradiation of 0.10 M nitrate, as a function of the concentration of added $NaHCO_3$. Irradiation was carried out under the Philips TL 01 lamp. Note the logarithmic scale and the break in the x-axis.