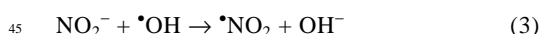# UNIVERSITÀ DEGLI STUDI DI TORINO

THEORETICAL AND EXPERIMENTAL EVIDENCE OF THE PHOTONITRATION PATHWAY OF PHENOL AND 4-CHLOROPHENOL: A MECHANISTIC STUDY OF ENVIRONMENTAL SIGNIFICANCE

5

Andrea Bedini,[a] Valter Maurino,[a] Claudio Minero,[a] Davide Vione[a] [b]*

Light-induced nitration pathways of phenols are important processes for the transformation of pesticide-derived secondary pollutants into toxic derivatives in surface waters, and for the

10 formation of phytotoxic compounds in the atmosphere. Moreover, phenols can be used as $^\bullet NO_2$ probes in irradiated aqueous solutions. This paper shows that the nitration of 4-chlorophenol (4CP) into 2-nitro-4-chlorophenol (NCP) in the presence of irradiated nitrate and nitrite in aqueous solution involves the radical $^\bullet NO_2$. The experimental data allow exclusion of an alternative nitration pathway by $^\bullet OH + {}^\bullet NO_2$. Quantum mechanical calculations suggest that the nitration of

15 both phenol and 4CP involves as a first pathway the abstraction of the phenolic hydrogen by $^\bullet NO_2$, which yields HNO_2 and the corresponding phenoxy radical. It follows reaction of phenoxyl with another $^\bullet NO_2$ to finally produce the corresponding nitrated phenol. Such a pathway also correctly predicts that 4CP undergoes nitration more easily than phenol, because the ring Cl atom increases the acidity of the phenolic hydrogen of 4CP. This favours the H-abstraction process to give the

20 corresponding phenoxy radical. In contrast, an alternative nitration pathway that involves $^\bullet NO_2$ addition to the ring followed by H-abstraction by oxygen (or by $^\bullet NO_2$ or $^\bullet OH$) is energetically unfavoured and erroneously predicts faster nitration for phenol than for 4CP.

Introduction

25 The occurrence of aromatic nitroderivatives in the environment is a matter of concern because they have toxic effects.[1] Some of these compounds may also act as inhibitors of phenol sulfotransferase enzymes, which are involved into detoxification processes.[2] Moreover, nitroaromatic

30 compounds can cause damage to DNA, which gives them potential for genotoxicity and mutagenicity.[3,4] Nitroaromatics can be directly emitted into the environment, but they are more often formed as secondary pollutants upon nitration of the corresponding aromatic precursors.[5,6,7] Nitration processes

35 can take place in the gas phase,[8,9] on particles [10] and in surface and atmospheric waters.[11,12,13] In the aqueous solution and in particular in surface waters, a large fraction of nitroaromatic formation is thought to take place *via* photonitration reactions.[14,15,16,17] Such processes are induced by irradiation of

40 nitrate and nitrite and involve at some level the photogeneration of $^\bullet NO_2$. The formation of $^\bullet NO_2$ by photolysis of nitrate and nitrite takes place as follows:[18]

$$NO_3^- + h\nu + H^+ \rightarrow {}^\bullet OH + {}^\bullet NO_2 \qquad (1)$$

$$NO_2^- + h\nu + H^+ \rightarrow {}^\bullet OH + {}^\bullet NO \qquad (2)$$

45 $$NO_2^- + {}^\bullet OH \rightarrow {}^\bullet NO_2 + OH^- \qquad (3)$$

The described photochemical reactions can play a comparable role as the nitrogen dioxide dissolution from the gas phase, as $^\bullet NO_2$ sources in atmospheric waters.[19] In this compartment, the formation of phenolic nitroderivatives in the presence of

50 $^\bullet NO_2$ is strongly suspected to substantially contribute to forest damage in polluted areas and in their downwind regions.[20]

Phenolic compounds are easily nitrated substrates and are the most studied chemicals as far as photonitration is concerned. It has for instance been shown that 4-chlorophenol

55 (4CP), an environmental transformation intermediate of the herbicide dichlorprop, can undergo photonitration into 2-nitro-4-chlorophenol (NCP) in paddy-field water.[16] Nevertheless, the actual mechanism of phenol photonitration has been the object of debate.[21,22] Elucidation of this reaction

60 pathway is very important for a better understanding of the environmental conditions that are most likely to favour the process. Moreover, the phenol photonitration reaction is a useful $^\bullet NO_2$ probe,[14,16] and the knowledge of the reaction mechanism is essential for a quantitative assessment of the

65 $^\bullet NO_2$ occurrence in irradiated systems.

In the literature many computational studies are present that describe the mechanism of benzene or phenol nitration via A_ES operated by nitronium ion,[23,24] because of the relevance of this reaction for organic synthesis and industrial

70 applications.[25,26,27] In contrast, little attention has been given to the nitration of phenolic compounds occurring via radical mechanisms,[28,29] because of the lower selectivity and of the difficulty to prepare large quantities of the reactant radical species. Because of the environmental interest of the

75 photonitration processes, the present study is focused on the understanding of the nitration mechanism of phenolic compounds in irradiated systems, using both quantum calculations and experimental evidence.

Experimental

Reagents and materials

4-Chlorophenol (purity grade 99%), 2-nitro-4-chlorophenol (98%), anisole (99%), 2-nitroanisole (99%), 3-nitroanisole (98%) and 4-nitroanisole (97%) were purchased from Aldrich, acetonitrile (gradient grade), $NaNO_3$ (99%) and H_3PO_4 (85%) from VWR Int., $NaNO_2$ (97%) from Carlo Erba. All reagents were used as received, without further purification.

Irradiation experiments

Solution to be irradiated (5 mL) were placed in cylindrical Pyrex glass cells (4.0 cm diameter, 2.3 cm height) and irradiated under magnetic stirring with a Philips TL 01 UVB lamp, with maximum emission at 313 nm. The lamp irradiance was 9.5 W m^{-2} (290-400 nm, measured with a power meter by CO.FO.ME.GRA., Milan, Italy) and the incident photon flux in solution was determined by ferrioxalate actinometry as

$$P_o = \int_\lambda p^\circ(\lambda)d\lambda = 6.2 \cdot 10^{-6}$$

Einstein L^{-1} s^{-1}. The lamp was chosen to achieve excitation of nitrate that absorbs radiation in the UVB region.[18]

After irradiation, the solutions were analysed by High Performance Liquid Chromatography (HPLC). The adopted Merck-Hitachi instrument was equipped with AS2000A autosampler (100 μL sample volume), 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 was set at 220 nm). Isocratic elution was carried out with 40:60 CH_3CN:aqueous H_3PO_4 (pH 2.8) at 1.0 mL min^{-1} flow rate. The retention times were (min): 4-chlorophenol (4CP, 4.2), 2-nitro-4-chlorophenol (NCP, 8.0), anisole (8.2), 2-nitroanisole (5.7), 3-nitroanisole (9.0), 4-nitroanisole (7.7). The column dead time was 0.9 min.

The time evolution curves of NCP were fitted with equations of the form $C^{NCP}_t = k^f_{NCP} C_o (k^d_{NCP} - k^d_{4CP})^{-1}$ $[exp(-k^d_{4CP} t) - exp(-k^d_{NCP} t)]$, where C^{NCP}_t is the concentration of NCP at the time t, C_o the initial concentration of 4CP, k^f_{NCP} and k^d_{NCP} the pseudo-first order formation and transformation rate constants of NCP, respectively, and k^d_{4CP} the pseudo-first order transformation rate constant of 4CP. The initial formation rate of NCP is $Rate_{NCP} = k^f_{NCP} C_o$. The reproducibility of repeated runs was around 10-15%.

Quantum mechanical calculations

A computational study of phenol and 4CP nitration mechanism was carried out using the density functional theory (DFT)[30]. DFT methods and in particular those using hybrid functionals have been widely employed to explore chemical reactions due to their good accuracy at a relatively low computational cost. They have been used for a large number of test molecules including radicals, non-hydrogen systems, aromatic and aliphatic hydrocarbons. In the present study, geometry optimisations of the ground state of all the involved species were carried out in the gas phase and were confirmed by analytical calculation of frequencies. For each molecular species, the gas-phase optimised structures were determined by gradient procedures [31] within DFT with the B3LYP hybrid functional.[32] The latter is a well-performing functional for reactions involving radical species and aromatic compounds.[33,34] The polarised 6-311G(d,p) basis set was used in all the calculations. The nature of the stationary points, minima or saddle points, was confirmed by analytical calculation of the vibrational frequencies to evaluate the corresponding zero-point vibrational energies. Calculations were performed in the gas phase and were not refined with any solvation model.

The reason of this approximation is that radical-forming reactions are usually not very sensitive to medium effects, because activated complexes that produce neutral radicals normally exhibit no charge separation. As a consequence, they have a minor ability to interact with the solvent compared to charged species.[35] Furthermore, very limited stabilisation energy or geometry changes induced by the solvent are expected in the present case, in analogy with the results of calculations performed on similar systems where radical reactions were taking place.[36,37,38] Finally, the main goal of this work is the comparison of two reaction pathways *via* the ratio of their rate constants, derived from the respective energy barriers. The limited solvation effects that would take place are expected to influence both pathways at a similar extent. The resulting small energy changes would be largely compensated for when making the ratio of the rate constants.

All calculations were carried out with the GAUSSIAN 03W suite of programs.[39] The figures were generated with the GaussView program.

Results and discussion

Photonitration of phenolic compounds upon irradiation of nitrate and nitrite

In the case of phenol photonitration upon irradiation of nitrate and/or nitrite, there is wide evidence that the reaction involves $^\bullet NO_2$ and not $^\bullet OH + {}^\bullet NO_2$.[40] A clear differentiation between the two possible pathways is to be provided for the photonitration of 4CP as well.

Figure 1 reports the initial formation rate of NCP as a function of the initial 4CP concentration, upon UVB irradiation of 0.10 M $NaNO_3$ + 0.01 M $NaNO_2$. The NCP rate follows an initially linear trend but starts levelling off above 0.1 mM 4CP.
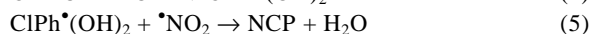
Upon application of the Lambert Beer's law to radiation absorption in mixtures under polychromatic irradiation (which involves procedures of numerical integration over wavelength),[41] one gets that the photon flux absorbed by 0.10 M nitrate in the system was $P_a^{NO3-} = 1.8 \cdot 10^{-6}$ Einstein L^{-1} s^{-1}. For 0.01 M nitrite it was $P_a^{NO2-} = 6.2 \cdot 10^{-7}$ Einstein L^{-1} s^{-1}.
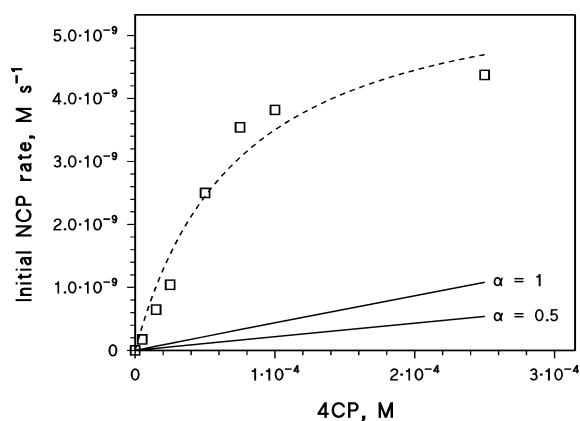
Figure 1. Initial formation rate of NCP as a function of 4CP initial concentration, upon UVB irradiation of 0.10 M $NaNO_3$ + 0.01 M $NaNO_2$ at pH 6 in aerated solution. The solid lines represent the predicted rates by equation (8), with the reported α values. The dashed curve is the fit of the experimental data with equation (13) (*vide infra*).

The quantum yields for $^\bullet OH$ formation by nitrate and nitrite under UVB irradiation are 0.01 and 0.065, respectively,[18] from which one gets the total $^\bullet OH$ formation rate in the system, $R_{\bullet OH} = R_{\bullet OH}^{NO3-} + R_{\bullet OH}^{NO2-} = 0.01\, P_a^{NO3-} + 0.065\, P_a^{NO2-} = 5.8 \cdot 10^{-8}$ Einstein L^{-1} s^{-1}. The presence of $^\bullet OH$ scavengers at sufficiently elevated concentration (*e.g.* 1mM or higher) could increase the photolysis quantum yield of nitrate by affecting the early steps after the primary photolysis event,[42,43] but this is unlikely to take place at a significant extent with 4CP at the adopted sub-mM levels.

If the nitration of 4CP takes place via $^\bullet OH$ + $^\bullet NO_2$, a kinetic model applies that is made up of reactions (1-3) plus the following ones (where ClPhOH is 4CP and 4CP-OH is an hydroxyderivative of 4CP):

$$ClPhOH + {}^\bullet OH \rightarrow ClPh^\bullet(OH)_2 \qquad (4)$$
$$ClPh^\bullet(OH)_2 + {}^\bullet NO_2 \rightarrow NCP + H_2O \qquad (5)$$
$$ClPh^\bullet(OH)_2 + O_2 \rightarrow 4CP\text{-}OH + HO_2^\bullet \qquad (6)$$
$${}^\bullet NO_2 \rightarrow Products \qquad (7)$$

In alternative to O_2, also $^\bullet OH$ and $^\bullet NO_2$ could take part in reaction (6) as hydrogen-abstracting reagents. As an approximated approach to solve the kinetic system, the lumped reaction (7) incorporates various transformation processes for nitrogen dioxide, including the reaction with $O_2^{-\bullet}$.[18] Upon application of the steady-state approximation to $^\bullet NO_2$, $^\bullet OH$ and $ClPh^\bullet(OH)_2$, one gets the following result for the initial formation rate of NCP:

$$d[NCP]/dt = \alpha \cdot R_{\bullet OH} \cdot \frac{k_4[4CP]}{k_4[4CP] + k_3[NO_2^-]} \qquad (8)$$

where $k_4 = 7.6 \cdot 10^9$ M^{-1} s^{-1} and $k_3 = 1.0 \cdot 10^{10}$ M^{-1} s^{-1}.[44] $\alpha = k_5$ $[^\bullet NO_2]$ $(k_5\,[^\bullet NO_2] + k_6\,[O_2])^{-1}$ is the fraction of the radical species $ClPh^\bullet(OH)_2$ that reacts with $^\bullet NO_2$ in reaction (5) to yield NCP, in competition with the reaction (6) with O_2 that produces the hydroxyderivative 4CP-OH. The NCP formation rates foreseen by equation (8) are reported as solid lines in Figure 1, for $\alpha = 1$ (reaction (5) much faster than (6)) and $\alpha = 0.5$ (equal rates for the two reactions). The equation (8) rates are considerably lower than the experimental ones, and one should also consider that the assumptions $\alpha = 1$ and $\alpha = 0.5$ most likely represent an overestimate of the NCP rate via the proposed $^\bullet OH$ + $^\bullet NO_2$ pathway. The formation rate of the hydroxyderivatives upon nitrate irradiation is in fact considerably higher than that of the nitroderivatives,[45] which suggests that reaction (5) would actually be much slower than (6). Therefore, the NCP formation rate via reactions (4-6) is expected to be even lower than is reported in Figure 1.

Figure 1 also shows that equation (8) foresees a linear trend with [4CP] of the NCP formation rate, in disagreement with the experimental data, because k_4 [4CP] « k_3 [NO_2^-]. The comparison between experimental data and model predictions clearly suggests that $^\bullet OH$ is involved at a minor to negligible extent in the nitration of 4CP. Therefore, the following discussion will only consider the possible pathways that may be involved in the nitration of phenol and of 4CP by $^\bullet NO_2$, excluding or neglecting the contribution of $^\bullet OH$ to the nitration process.

Nitration pathways of phenolic compounds by $^\bullet NO_2$

Two alternative mechanisms have been proposed for the photonitration of phenol by $^\bullet NO_2$,[21,22] which are reported in Scheme 1. We hypothesise the same processes for the photonitration of 4CP as well. Furthermore, because 4CP can only be nitrated in the *ortho* position with respect to the phenolic group, the *para* one being occupied by Cl, only the *ortho* nitration pathway is shown in Scheme 1.
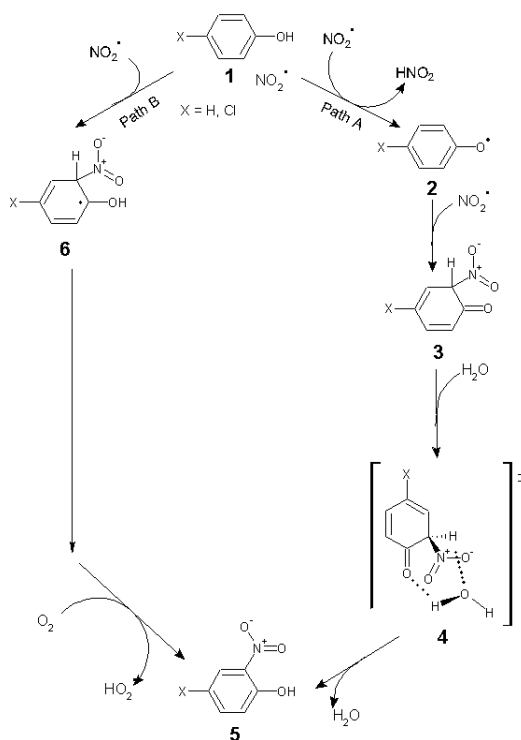
Scheme 1. Reaction of nitrogen dioxide with phenol and 4CP *via* radical pathways.

The first mechanism (Path A) involves as primary step an hydrogen abstraction by $^\bullet NO_2$ from the phenolic OH group. In contrast, the second mechanism (Path B) foresees addition of nitrogen dioxide to the aromatic ring.

5   Path A starts with a H-transfer redox reaction that involves $^\bullet NO_2$ as the oxidant and phenol as the reductant. Such a process yields the phenoxyl radical **2** and nitrous acid. It follows addition of $^\bullet NO_2$ to **2** with production of **3**. In this pathway, the rearomatisation of **3** gives the 2-nitrophenol **5** 10 through a hydrogen rearrangement catalysed by a water molecule. This step is likely to involve the transient **4**, as proposed by Houk for tyrosine nitration by peroxynitrite.[46]

In the alternative Path B, the addition of $^\bullet NO_2$ to the aromatic ring generates the intermediate **6**. The 2-nitrophenol **5** is 15 formed from **6** by H abstraction, which restores the aromaticity of the ring. H abstraction can be carried out by dissolved O_2 to produce the hydroperoxyl radical $HO_2{}^\bullet$; alternative reagents for the process are $^\bullet NO_2$ (which yields HNO_2) and $^\bullet OH$ that yields H_2O.

20   In the following section we undertake the comparison of the energetics of the nitration processes of phenol and 4CP. The elucidation of the 4CP pathway is particularly interesting because it has been found that 4CP photonitration is faster than that of phenol in the aqueous solution.[16]

25 ## Modelling of the reaction between $^\bullet NO_2$ and phenolic compounds

To get further insight into the different reactivity of the studied compounds, a DFT quantum mechanical study was carried out of the ground states of phenol and 4CP. Geometry 30 optimisations and spin distributions of the corresponding phenoxy radicals are reported in ESI. As already mentioned, 4CP only undergoes *ortho* nitration and, to achieve a better comparison between the two substrates, in the case of phenol only the path that yields 2-nitrophenol was considered. An 35 analogous pathway will also yield 4-nitrophenol.

Nitration of phenol by $^\bullet NO_2$

The first mechanism (Path A in Scheme 1) involves first hydrogen abstraction by $^\bullet NO_2$, followed by addition of another $^\bullet NO_2$ to the generated phenoxyl radical **2** to give **3**. A 40 water molecule assists the rearomatisation of the ring to give **5,** through an arrangement of a hydrogen atom in *ortho* to the quinonic oxygen of **3**. In the case of phenol, the transition state **4** for this rearomatization step is depicted in Figure 2.

The other mechanism (Path B, Scheme 1) involves $^\bullet NO_2$ 45 addition to **1** to give the intermediate radical **6**. In this study we have considered the further reaction of **6** with oxygen, which restores aromaticity upon hydrogen abstraction and yields **5**. Alternative or additional reactants ($^\bullet NO_2$, $^\bullet OH$) could also be involved in the H-abstraction process.

50   Figure 3 shows the free energy profiles of the reactions of $^\bullet NO_2$ with phenol via Path A and Path B. The calculated energy barriers for the first reaction steps are 19 kcal/mol and 27 kcal/mol for H-abstraction and $^\bullet NO_2$ addition, respectively.
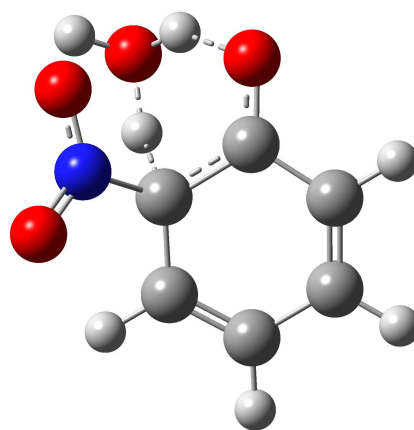
55

Figure 2. Transition state (TS) **4** for the water-assisted rearomatisation process of intermediate **3** to give product **5**.
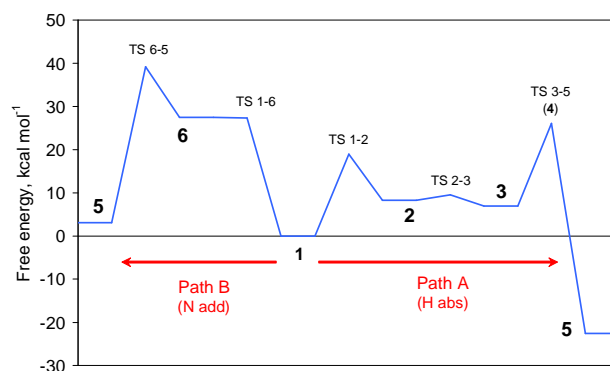
Figure 3. Free energy diagram for the reaction of phenol with 60 nitrogen dioxide via two possible pathways: hydrogen abstraction (H abs, *i.e.* Path A) and nitrogen dioxide addition (N add, *i.e.* Path B).

By using the well-known Eyring formula [47] within the Transition State Theory (TST) and by comparing the two 65 energy barrier values, one sees a difference of 6 orders of magnitude between the kinetic constants of the two processes, which makes the $^\bullet NO_2$ addition to the aromatic ring quite unlikely.

Furthermore, the radical intermediate labelled as **6** has very 70 high energy compared to the TS. This increases the probability that $^\bullet NO_2$ gets away from the ring and goes back to the solution, giving back **1** with a process that has almost no energy barrier. Interestingly, such a conclusion can be reached by considering only the first reaction step, 75 irrespective of the fact that the radical **6** undergoes reaction with O_2, $^\bullet NO_2$ or $^\bullet OH$ to finally yield **5**.

In the subsequent steps of Path A, the reaction of **2** with a second $^\bullet NO_2$ to give **3** is almost barrierless. Indeed, it is a coupling reaction between two radicals and proceeds in 80 solution at a diffusion-controlled rate. The reaction of rearomatisation of **3** through the TS **4** to give **5** has an energy barrier of 19 kcal/mol and is quite exothermic (49 kcal/mol).

These calculated values are in agreement with other values reported in the literature for the ONOO⁻−mediated nitration of tyrosine.[46]

In the case of Path B, after the formation of the intermediate **6,** there is involvement of oxygen in its triplet state that abstracts a hydrogen and re-establishes aromaticity in the ring by forming **5**. This step has an energy barrier of 12 kcal/mol. However, even in the presence of a lower energy barrier that could be afforded by another H-abstracting reactant, Path B would be considerably more difficult than A. Another interesting issue is that **6** is quite likely to give back **1** because the corresponding process is barrierless. In contrast, in Path A, the back transformation of **2** into **1** would be made more difficult by an energy barrier, thereby enabling the process to also proceed toward **5**.

Nitration of 4CP by $^{\bullet}NO_2$

Figure 4 shows the free energy profiles of the reaction of $^{\bullet}NO_2$ with 4CP via the usual Path A and Path B. The overall profiles are quite similar to those of phenol and, also in this case, it can be clearly seen that Path A (hydrogen abstraction by $^{\bullet}NO_2$, followed by addition of a second $^{\bullet}NO_2$ to the phenoxy radical) is much favored compared to Path B (addition of $^{\bullet}NO_2$ to the aromatic ring).
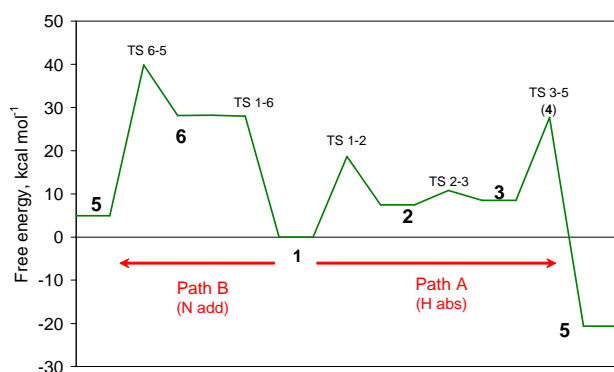
Figure 4. Free energy diagram for the reaction of 4-chlorophenol (4CP) with nitrogen dioxide via two pathways: hydrogen abstraction (H abs, *i.e.* Path A) and nitrogen dioxide addition (N add, *i.e.* Path B).

Comparison of the reaction pathways

The quantum mechanical calculations concerning the nitration of phenol and of 4CP show that Path A, involving an initial H abstraction by $^{\bullet}NO_2$ on the phenolic group to give the phenoxy radical is strongly favoured over Path B, which proceeds via addition of $^{\bullet}NO_2$ to the ring. This computational result is supported by additional experimental evidence, concerning in particular the inability of anisole to undergo photonitration in the presence of either nitrate or nitrite under irradiation, or with their mixture (data not shown). The absence of anisole photonitration can be easily explained in the context of Path A, because the phenolic hydrogen that is involved in the first step of the nitration process is replaced by a methyl group.

By comparing the free energy diagrams relative to Path A

and reported in Figure 3 (phenol) and in Figure 4 (4CP), it can be observed that the difference in terms of energy barriers among the two transition states (TSs) is very low (~0.3 kcal/mol). However, the TS of 4CP is a bit lower in energy than the phenol one, suggesting that the nitration process could be faster with 4CP as substrate, in agreement with the experimental data.[16] This finding could be explained by the inductive effect of the Cl atom, which recalls electron density from the ring and makes the phenolic hydrogen more acidic and, therefore, more liable to be detached (see ESI for details about the spin distribution and for the direct comparison of the two reaction pathways). Indeed, 4CP has a pK_a value of 9.43, to be compared with $pK_a = 9.99$ for phenol.[48,49]

Interestingly, in the case of Path B the energy barrier of the first reaction step ($^{\bullet}NO_2$ addition to the ring) is slightly lower, by 0.7 kcal mol⁻¹, for phenol than for 4CP. This probably happens because the inductive effect of the 4CP chlorine atom in the *para* position reduces the ring electron density, thereby inhibiting the ring addition of radicals having an electrophilic character, such as $^{\bullet}NO_2$. This means that, according to Path B, the nitration of phenol into 2-nitrophenol should be faster than that of 4CP, which is not in agreement with the experimental results.[16]
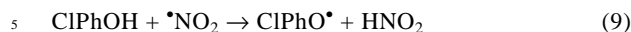
The fact that Path A predicts the correct reactivity order of phenol and 4CP toward nitration, differently from Path B, constitutes additional evidence that the photonitration of both compounds involves H abstraction by $^{\bullet}NO_2$ as the first reaction step.

It is also possible to derive within the TST the microkinetic constants of H-abstraction from the values of the energy barriers.[47] The calculated rate constant ratio k_{4CP}/k_{phenol} is 1.7. By assuming, as derived in the present paper, that $^{\bullet}NO_2$ is involved in the photonitration of both phenol and 4CP, the experimental data give a ratio of 5.8.[16] The fact that theoretical calculations are able to correctly predict at least the order of magnitude of the ratio is a good result, but it should be reminded that it is very risky to make reasonable scientific speculations on such small energy differences (< 2 kcal/mol) as have been observed between the TSs of phenol and of 4CP. Indeed, it is not possible to ignore the errors caused by both the basis set/functional used and the TST, which would not be very far from the energy differences involved.[50]

Note that path A predicts the formation of HNO₂ in its first step, and HNO₂ could be easily oxidised to $NO_3^- + H^+$ under the adopted photochemical conditions. However, path B yields $HO_2^{\bullet}$ ($pK_a = 4.8$ [51]) that would be readily deprotonated to $O_2^{-\bullet} + H^+$ at the adopted initial pH of 6. Therefore, path A and path B would cause a comparable acidification. More importantly, acidification from the cited processes would be largely overcome by the consumption of H^+ upon photolysis of nitrate and nitrite (reactions 1,2). These issues prevent the experimental demonstration of the occurrence of path A by direct pH measurements.

Comparison with the experimental data

A 4CP nitration mechanism that follows Path A would involve the following reactions, where ClPhOH is 4CP and ClPhO$^\bullet$ is the corresponding phenoxy radical:

5   $ClPhOH + {}^\bullet NO_2 \rightarrow ClPhO^\bullet + HNO_2$ (9)
   $ClPhO^\bullet + {}^\bullet NO_2 \rightarrow NCP$ (10)
   $ClPhO^\bullet \rightarrow Products$ (11)
   ${}^\bullet NO_2 \rightarrow Products$ (12)

The lumped reactions (11,12) include several transformation

10 pathways of ClPhO$^\bullet$ and ${}^\bullet NO_2$, among which are the reactions with superoxide.[18,45] The formation of ${}^\bullet NO_2$ takes place via reactions (1) and (3) and, because $R_{{}^\bullet OH}{}^{NO3-} = R_{{}^\bullet NO2}{}^{NO3-}$, it is $R_{{}^\bullet NO2} = R_{{}^\bullet OH}{}^{NO3-} + k_3\,[{}^\bullet OH]\,[NO_2^-]$, with $[{}^\bullet OH] = R_{{}^\bullet OH}\,(k_4$ $[4CP] + k_3\,[NO_2^-])^{-1}$. Upon application of the steady-state

15 approximation to ClPhO$^\bullet$ and to ${}^\bullet NO_2$ one gets the following expression for the NCP formation rate:

$$\frac{d[NCP]}{dt} = \left(R_{{}^\bullet OH}^{NO_3^-} + R_{{}^\bullet OH} \cdot \frac{k_3[NO_2^-]}{k_3[NO_2^-] + k_4[4CP]} \right) \cdot \frac{k_9[4CP]}{k_9[4CP] + k_{12}} \cdot \beta$$

(13)

where $\beta = k_{10}\,[{}^\bullet NO_2]\,(k_{10}\,[{}^\bullet NO_2] + k_{11})^{-1}$ is the fraction of

20 ClPhO$^\bullet$ that reacts with ${}^\bullet NO_2$ to yield the 4CP. With the known values of $R_{{}^\bullet OH}{}^{NO3-}$, $R_{{}^\bullet OH}$, k_3 and k_4 it is possible to fit the experimental data of NCP formation rate with equation (13), obtaining $\beta = 0.082$ and $k_{12}\,k_9^{-1} = 7.7 \cdot 10^{-5}$ M. The fit is shown as the dashed curve in Figure 1 and it is in good agreement with the

25 experimental data ($R^2 = 0.97$).

Conclusions

The experimental data reported in this paper allow exclusion of a 4CP nitration process that takes place via ${}^\bullet OH + {}^\bullet NO_2$. The same process could be excluded for the photonitration of

30 phenol.[40] In contrast, nitration of both phenolic compounds should involve ${}^\bullet NO_2$ alone. The are two possible pathways that could account for the nitration of phenol and of 4CP by ${}^\bullet NO_2$. Path A proceeds via H abstraction by ${}^\bullet NO_2$ on the phenolic oxygen to give HNO$_2$ and the corresponding

35 phenoxy radical. The latter reacts with another ${}^\bullet NO_2$ to finally yield the nitrophenol. Path B involves addition of ${}^\bullet NO_2$ to the aromatic ring, followed by H abstraction operated by O$_2$, ${}^\bullet NO_2$ or ${}^\bullet OH$ to give the nitrophenol. Quantum mechanical calculations, carried out following the DFT theory indicate

40 that path A would be strongly favoured over B because of the much lower activation energy barrier. Moreover, the energetics of path B suggests that the reaction intermediate would be decomposed back to the reactants far more easily than it can evolve into the nitrophenol product. Finally, path

45 A correctly predicts that the nitration of 4CP is faster than that of phenol,[16] because the chlorine substituent on the ring makes the phenolic hydrogen more acidic and favours the formation of the phenoxy radical. An additional issue is the lack of anisole nitration in the presence of nitrate and/or

50 nitrite under irradiation. It is consistent with the occurrence of path A, because in anisole the phenolic hydrogen is replaced by a methyl group. Nitration of 4CP *via* Path A also allows

the elaboration of a kinetic model that is in good agreement with the experimental data.

55   Nitration of phenolic compounds would thus involve two ${}^\bullet NO_2$ radicals, which has implications for the use of phenol as a probe molecule for nitrogen dioxide. Indeed, phenol photonitration can be adopted to derive the formation rate of nitrogen dioxide in an irradiated sample. The ${}^\bullet NO_2$ formation

60 rate calculated under the hypothesis that two ${}^\bullet NO_2$ are involved in nitration is exactly the double than for the case where nitration involves just one ${}^\bullet NO_2$. Therefore, the involvement of two ${}^\bullet NO_2$ in phenol photonitration *via* Path A should be taken into account when using such a reaction as a

65 ${}^\bullet NO_2$ probe in solution.

Notes and references

[a] *Dipartimento di Chimica Analitica, Università di Torino, Via P. Giuria 5, 10125 Torino, Italy. http://www.chimicadellambiente.unito.it. Fax: +39-011-6707615; E-mail: davide.vione@unito.it*

75 [b] *Centro Interdipartimentale NatRisk, Via Leonardo da Vinci 44, 10095 Grugliasco (TO); Italy. http://www.natrisk.org*
† Electronic Supplementary Information (ESI) available: xxx. See DOI:

1 A. Tognazzi, A.M. Dattilo, L. Bracchini, C. Rossi and D. Vione, Chemical characterisation of a new estuarine pollutant (2,4-dichloro-1 6-nitrophenol) and assessment of the acute toxicity of its quinoid form for *Artemia salina*, *Intern. J. Environ. Anal. Chem.*, accepted.

2 M.B. Kroegerkoepte, S.R. Koepte, L. Hernandez and C.J. Michejda, activation of a β-hydroxylkylnitrosamine by alkylating agents – Evidence for the involvement of a sulfotransferase, *Cancer Res.*, 1992, **52**, 3300-3305.

3 S. Chiron, S. Barbati, M. De Méo, A. Botta, In vitro synthesis of 1,N⁶-etheno-2′-deoxyadenosine and 1,N²-etheno-2′-deoxyguanosine by 2,4-dinitrophenol and 1,3-dinitropyrene in presence of a bacterial nitroreductase, *Environ. Toxicol.*, 2007, **22**, 222-227.

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

5 J.N. Pitts Jr., J. Arey, B. Zielinska, A.M. Winer and R. Atkinson, Determination of 2-nitrofluoranthene and 2-nitropyrene in ambient particulate organic matter: Evidence for atmospheric reactions, *Atmos. Environ.*, 1985, **19**, 1601-1608.

6 P. Ciccioli, A. Cecinato, E. Brancaleoni, M. Frattoni, P. Zacchei, A.H. Miguel and P. De Castro Vasconcellos, Formation and transport of 2-nitrofuoranthene and 2-nitropyrene of photochemical origin in the troposphere, *J. Geophys. Res.*, 1996, **101**, 19567-19581.

7 M. Vincenti, V. Maurino, C. Minero and E. Pelizzetti, Detection of nitro-substituted polycyclic aromatic hydrocarbons in the Antarctic airborne particulate, *Intern. J. Environ. Anal. Chem.*, 2001, **79**, 257-272.

8 R.I. Olariu, B. Klotz, I. Barnes, K.H. Becker and R. Mocanu. Ft-IR study of the ring-retaining products from the reaction of ${}^\bullet OH$ radicals with phenol, o-, m- and p-cresol. *Atmos. Environ.*, 2002, **36**, 3685-3697.

9 R.I. Olariu, I. Bejan, I. Barnes, B. Klotz, K.H. Becker and K. Wirtz, Rate coefficients for the gas-phase reaction of NO$_3$ radicals with selected dihydroxybenzenes, *Int. J. Chem. Kinet.*, 2004, **36**, 577-583.

10 T. Ramdahl, A Bjørseth, D.M. Lokensgard and J.N. Pitts Jr., Nitration of polycyclic aromatic hydrocarbons adsorbed to different carriers in a fuidized bed reactor, *Chemosphere*, 1984, **13**, 527-534.

11 J. Lüttke, V. Schher, K. Levsen, G. Wöünsch, J N. Cape, K. J. Hargreaves, R. L. Storeton-West, K. Acker, W. Wieprecht and B.

Jones, Occurrence and formation of nitrated phenols in and out of cloud, *Atmos. Environ.*, 1997, **31**, 2637-2648.

12 D. Vione, V. Maurino, C. Minero and E. Pelizzetti, 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

13 M. A. J. Harrison, M. R. Heal and J. N. Cape, Evaluation of the pathways of troposhperic nitrophenol formation from benzene and phenol using a multiphase model, *Atmos. Chem. Phys.*, 2005, **5**, 1679-1695

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

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

16 P.R. Maddigapu, D. Vione, B. Ravizzoli, C. Minero, V. Maurino, L. Comoretto and S. Chiron, Laboratory and field evidence of the photonitration of 4-chlorophenol to 2-nitro-4-chlorophenol and of the associated bicarbonate effect, *Environ. Sci. Pollut. Res.*, 2010, **17**, 1063-1069.

17 P.J. Squillace, J.C. Scott, M.J. Moran, B.T. Nolan and D.W. Kolpin, VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States, *Environ. Sci. Technol.*, 2002, **36**, 1923–1930

18 J. Mack and J.R. Bolton, Photochemistry of nitrite and nitrate in aqueous solution: A review, *J. Photochem. Photobiol. A: Chem.*, 1999, **128**, 1-13.

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

20 M. A. J. Harrison, S. Barra, D. Borghesi, D. Vione, C. Arsene and R. I. Olariu, *Atmos. Environ.*, 2005, **39**, 231-248.

21 R. Louw and D. Santoro, D., 1999. Comment on Formation of nitroaromatic compounds in advanced oxidation processes: photolysis versus photocatalysis, Environ. Sci. Technol., 1999, 33, 3281.

22 J. Dzengel, J. Theurich and D.W. Bahnemann, Response to comment on Formation of nitroaromatic compounds in advanced oxidation processes: photolysis versus photocatalysis. *Environ. Sci. Technol.*, 1999, **33**, 3282.

23 L. Chen, H. Xiao, J. Xiao and X. Gong, DFT Study on nitration mechanism of benzene with nitronium ion, *J. Phys. Chem. A*, 2003, **107**, 11440–11444.

24 J. Freire de Queiroz, J. Walkimar de M. Carneiro, A.A. Sabino, R. Sparrapan, M.N. Eberlin and P.M. Esteves Electrophilic aromatic nitration: understanding its mechanism and substituent effects *J. Org. Chem.*, 2006, **71**, 6192–6203

25 Kent and Riegel's Handbook of industrial chemistry and biotechnology 11th ed., Springer 2007, 1793 pp.

26 M. Baudouin, J.-L. Bougeois, S. Ratton and J.-P. Lecouve, Patent US 5030769, 1991,Continuous direct preparation of nitrophenols.

27 L. Ducry, D.M. Roberge, Controlled Autocatalytic Nitration of Phenol in a Microreactor *Angewandte Chemie* 2005, **117**, 8186–8189

28 M.J. Thompson and P.J. Zeegers, A theoretical study on the two-phase nitration of phenols *Tetrahedron* 1989, **45**, 191-202; M.J. Thompson and P.J. Zeegers Studies on the two-phase nitration of phenols (part 2) *Tetrahedron* 1990, **46**, 2661-2674

29 R.J. Coombes and A.W. Diggle The mechanism of nitration of phenol and 4-methylphenol by nitrogen dioxide in solution *Tetrahedron Lett.* 1991, **47**, 8787-8790

30 W. Kohn and L. J. Sham, Self-consistent equations including exchange and correlation effects, *Phys. Rev.*, 1965, **140**, A1133-1138.

31 J. B. Foresman and Æ. Frisch, *Exploring Chemistry with Electronic Structure Methods*, Gaussian, Inc., Pittsburgh, PA, 1996, pp. 166-168.

32 A. D. Becke, Density-functional thermochemistry. III. The role of exact exchange, *J. Chem. Phys.*, 1993, **98**, 5648-5652.

33 Y. Zhang and K. Huang DFT studies on the role of glutamate residue in the tyrosine nitration *J. Mol. Str. Theochem*, 2008, **864**, 48–55

34 H. Roohi, DFT study of the biphenylene–NO$_2$$^+$ complexes formed in nitration mechanism, *J. Phys. Org. Chem.*, 2008, **21**, 971–978

35 C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry, 3rd Edition*, Wiley-VCH, Weinheim, 2003.

36 M. Kiliç and Z. Çinar,   Hydroxyl radical reactions with 4-chlorophenol as a model for heterogeneous photocatalysis, *J. Mol. Struc. – Theochem*, 2008, **851**, 263-270.

37 M. Kiliç, G. Koçtürk, N. San and Z. Çinar, A model for prediction of product distributions for the reactions of phenol derivatives with hydroxyl radicals, *Chemosphere*, 2007, **69**, 1396–1408.

38 A. Delabie, S. Creve, B. Coussens and M.T. Nguyen, Theoretical study of the solvent effect on the hydrogen abstraction reaction of the methyl radical with hydrogen peroxide, *J. Chem. Soc., Perkin Trans. 2*, 2000, 977–981

39 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle and J. A. Pople, *Gaussian 03, revision B.05*, Gaussian, Inc., Pittsburgh, PA, 2003.

40 D. Vione, V. Maurino, C. Minero, D. Borghesi, M. Lucchiari and E. Pelizzetti, New processes in the environmental chemistry of nitrite. 2. The role of hydrogen peroxide, *Environ. Sci. Technol.*, 2003, **37**, 4635-4641.

41 P.R. Maddigapu, C. Minero, V. Maurino, D. Vione, M. Brigante, T. Charbouillot, M. Sarakha and G. Mailhot, Photochemical and photosensitised reactions involving 1-nitronaphthalene and nitrite in aqueous solution, *Photochem. Photobiol. Sci.*, 2011, **10**, 601-609.

42 P. Nissenson, D. Dabdub, R. Das, V. Maurino, C. Minero and D. Vione, Evidence of the water-cage effect on the photolysis of NO$_3$$^-$ and FeOH^{2+}. Implications of this effect and of H$_2$O$_2$ surface accumulation on photochemistry at the air-water interface of atmospheric droplets, *Atmos. Environ.*, 2010, **44**, 4859-4866.

43 D. Vione, B. Sur, B. K. Dutta, V. Maurino, and C. Minero, On the effect of 2-propanol on phenol photonitration upon nitrate photolysis, J. Photochem. Photobiol. A: Chem., in press. DOI: 10.1016/j.jphotochem.2011.09.008.

44 G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, Critical review of rate constants for reactions of hydrated electron, hydrogen atoms and hydroxyl radicals ($^\bullet$OH/$^\bullet$O$^-$) in aqueous solution, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513-886.

45 D. Vione, V. Maurino, C. Minero and E. Pelizzetti, Reactions induced in natural waters by irradiation of nitrate and nitrite ions, in: P. Boule, D.W. Bahnemann and P.K.J. Robertson (eds.), The Handbook of Environmental Chemistry vol. 2.M (Environmental Photochemistry part II), Springer, Berlin, 2005, pp. 221-253.

46 H. Gunaydin and K.N. Houk, Mechanisms of peroxynitrite-mediated nitration of tyrosine, *Chem. Res. Toxicol.*, 2009, **22**, 894–898.

47 H. Eyring, The activated complex in chemical reactions *J. Chem. Phys.*, 1935, **3**, 107-115.

48 L. Lepri, P. Desideri and D. Heimler, Reversed-phase and soap thin-layer chromatography of phenols. *J. Chromatogr. A*, 1980, **195**, 339-348.

49 A.O. Aptula, T.I. Netzeva, I.V. Valkova, M.T.D. Cronin, T.W. Schultz, R. Kühne and G. Schüürmann, Multivariate discrimination between modes of toxic action of phenols, *Quant. Struct.-Act. Relat.*, 2002, **21**, 12-22.

50 S.S. Shaik, H.B. Schlegel and S. Wolfe, Theoretical aspects of physical organic chemistry: The Sn2 mechanism, Wiley-Interscience 1991, 304 pp.

51 P. Warneck and C. Wurzinger, Product quantum yields for the 305-nm photodecomposition of NO_3^- in aqueous solution, *J. Phys. Chem.*, 1988, **92**, 6278-6283.