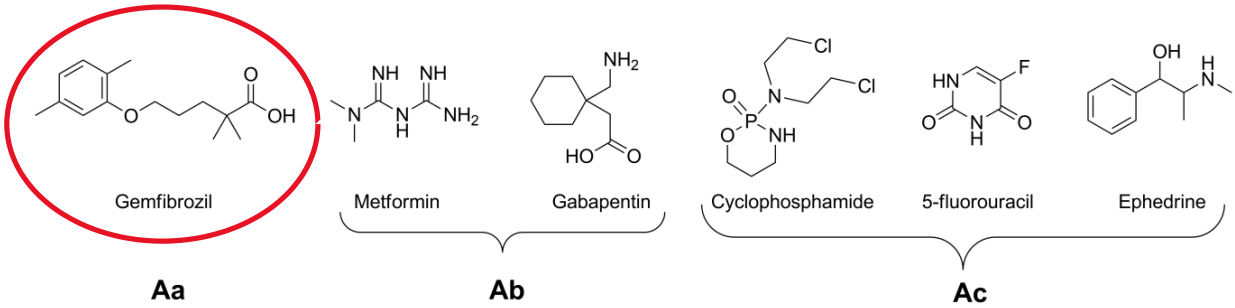
PHARMA-ECO *HRZZ project IP-2022-10-2634*

A report on

D1. Quantitative/qualitative description of results for chlorination and irradiation of selected (candidates according to the pillar Aa) pharmaceuticals, assignation of products in the reaction mixture; report published at the project web-pages (linked to O1-O3)

Antonio Ljulj, Moises Canle, Dean Marković, Maria Kolympadi Markovic, Valerije Vrček

The case of gemfibrozil

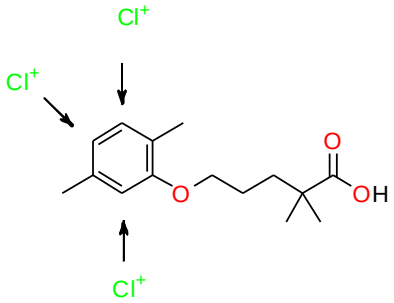


Scheme. Selected pharmaceuticals with the corresponding pillars, Aa, Ab or Ac, on which their definition as candidates for the 4th Watch List is based.

The selected drugs suitable for research within the framework of this project comply with the pillars and priority criteria set by the competent authorities. The group of candidates consists of more than 30 drugs listed in the technical report (Gomez Cortes, L.; Marinov, D.; Sanseverino, I.; Navarro Cuenca, A.; Niegowska Conforti, M.; Porcel Rodriguez, E.; Stefanelli, F.; Lettieri, T. Selection of substances for the 4th Watch List under the Water Framework Directive, Publications Office of the European Union, Luxembourg, **2022**.): the group of benzimidazoles, mebendazole, gemfibrozil and synthetic hormones are in accordance with the pillar **Aa** (the outcome of the risk assessment for the drug from the third monitoring list).

Most likely reactive sites on gemfibrozil (site-mapping)

Site A (dominant): Aromatic ring on the phenoxy moiety
Gemfibrozil contains a 2,5-dimethylphenoxy ring. The phenoxy (–O–) substituent is strongly activating toward electrophilic aromatic substitution (EAS), and methyl groups are weak activators; therefore, ring chlorination is the primary expected transformation class under HOCl-driven conditions.



Site B (secondary): Benzylic/alkyl side chain (oxidation)
Oxidation/hydroxylation on the alkyl chain can occur, but in "chlorine-only" disinfection it is typically secondary to rapid ring substitution, unless contact times are long or the matrix generates reactive chlorine radicals (this is more relevant to UV/chlorine AOP than to chlorination alone).

Site C (generally not primary): Carboxylic acid
Direct "chlorination" at the carboxyl group is not generally the dominant pathway in aqueous free chlorine disinfection for such structures; it is better treated as a functionality affecting speciation and partitioning rather than a principal electrophilic target.

Chlorination of Gemfibrozil (literature review)

Only monochlorination product considered!

Environmental Science & Technology > Vol 46/Issue 10 > Article

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ARTICLE | April 12, 2012

Analytical and Biological Characterization of Halogenated Gemfibrozil Produced through Chlorination of Wastewater

Daryl N. Bulloch[†], Ramon Lavado[§], Kristy L. Forsgren[§], Szabolcs Beni[†‡], Daniel Schlenk[§*], and Cynthia K. Larive[†*]

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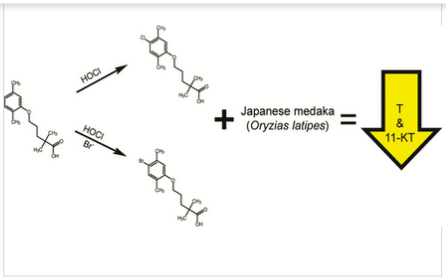
environmental contaminant because of its frequency of detection in U.S. wastewaters at concentrations which have been shown to disrupt endocrine function in aquatic species. The treatment of gemfibrozil solutions with sodium hypochlorite yielded a 4'-chlorinated gemfibrozil analog (chlorogemfibrozil). In the presence of bromide ion, as is often encountered in municipal wastewater, hypobromous acid generated through a halogen exchange reaction produced an additional 4'-brominated gemfibrozil product (bromogemfibrozil). Standards of chloro- and bromogemfibrozil were synthesized, isolated and characterized using mass spectrometry and NMR spectroscopy. Mass spectrometry was used to follow the in situ halogenation reaction of gemfibrozil in deionized water and wastewater matrices, and to measure levels of gemfibrozil (254 ± 20 ng/L), chlorogemfibrozil (166 ± 121 ng/L), and bromogemfibrozil (50 ± 11 ng/L) in advanced primary wastewater treatment effluent treated by chlorination. Chlorogemfibrozil demonstrated a significant ($p < 0.05$) reduction in the levels of 11-ketotestosterone at 55.1 μg/L and bromogemfibrozil demonstrated a significant ($p < 0.05$) reduction in the levels of testosterone at 58.8 μg/L in vivo in Japanese medaka in a 21 day exposure. These results indicated that aqueous exposure to halogenated degradates of gemfibrozil enhanced the antiandrogenicity of the parent compound in a model fish species, demonstrating that chlorination may increase the toxicity of pharmaceutically active compounds in surface water.



Chlorination Reactions. In situ chlorination reactions were performed using either Millipore deionized water or blended primary/secondary final effluent from Wastewater A. A 13% active chlorine solution was used for the introduction of sodium hypochlorite. Gemfibrozil was added to stirred deionized water or wastewater effluent solutions from a stock solution in methanol to effect a 100 μg/L concentration, with methanol accounting for no more than 0.1% of the overall reaction volume. At various time points, 500 μL of the reaction medium was sampled to determine product formation using a Waters (Milford, MA) Acquity ultra performance liquid chromatograph with a quadrupole time-of-flight mass spectrometric (UPLC-QTOF) detection (see SI for method details).

Environmental Science & Technology > Vol 54/Issue 18 > Article

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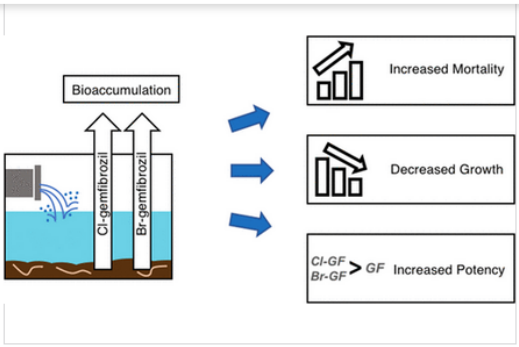
CONTAMINANTS IN AQUATIC AND TERRESTRIAL ENVIRONMENTS | August 14, 2020

Novel Disinfection Byproducts Formed from the Pharmaceutical Gemfibrozil Are Bioaccumulative and Elicit Increased Toxicity Relative to the Parent Compound in Marine Polychaetes (*Neanthes arenaceodentata*)

Nicolette E. Andrzejczyk*, Justin B. Greer, Eric Nelson, Junqian Zhang, John M. Rimoldi, Rama S. V. Gadepalli, Isaiah Edwards, and Daniel Schlenk

Formation of halogenated disinfection byproducts (DBPs) from pharmaceutically active compounds has been observed in water supply systems following wastewater chlorination. Although research has been limited thus far, several studies have shown that halogenated DBPs may elicit increased toxicity compared to their parent compounds. For example, the lipid regulator gemfibrozil has been shown to form chlorogemfibrozil (Cl-gemfibrozil) and bromogemfibrozil (Br-gemfibrozil) following chlorination, which are more potent antiandrogens in male Japanese medaka (*Oryzias latipes*) compared to their parent compounds. In the present study, we aimed to characterize the bioaccumulative ability of halogenated gemfibrozil DBPs in marine polychaetes *via* chronic sediment exposures and, consequently, to assess the chronic and acute toxicity of halogenated gemfibrozil DBPs through sediment (*in vivo*) and aqueous (*in vivo* and *in silico*) toxicity evaluations. Following 28 day sediment exposures, Cl-gemfibrozil and Br-gemfibrozil bioaccumulated within *Neanthes arenaceodentata*, with both compounds reducing survival and growth. The biota–sediment accumulation factors determined for Cl-gemfibrozil and Br-gemfibrozil were 2.59 and 6.86, respectively. Furthermore, aqueous 96 h toxicity tests with *N. arenaceodentata* indicated that gemfibrozil DBPs elicited increased toxicity compared to the parent compound. While gemfibrozil had an acute LC50 value of 469.85 ± 0.096 mg/L, Cl-gemfibrozil and Br-gemfibrozil had LC50 values of 12.34 ± 0.085 and 9.54 ± 0.086 mg/L, respectively. Although acute toxicity is relatively low, our results indicate that halogenated gemfibrozil DBPs are bioaccumulative and may elicit effects in apex food web organisms prone to accumulation following lifelong exposures.



Mono-, di-, and tri-chlorination products considered!

WATER RESEARCH 45 (2011) 1414–1422

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Identification of reaction products from reactions of free chlorine with the lipid-regulator gemfibrozil

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ABSTRACT

High global consumption rates have led to the occurrence of pharmaceutically active compounds (PhACs) in wastewater. The use of chlorine to disinfect wastewater prior to release into the environment may convert PhACs into uncharacterized chlorinated by-products. In this investigation, chlorination of a common pharmaceutical, the antihyperlipidemic agent gemfibrozil, was documented. Gemfibrozil (2,2-dimethyl-5-(2,5-dimethylphenoxy)pentanoic acid) was reacted with sodium hypochlorite and product formation was monitored by gas chromatography-mass spectrometry (GC-MS). The incorporation of one, two or three chlorine atoms into the aromatic region of gemfibrozil was demonstrated using negative-ion electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS). Further analysis using ^{1}H nuclear magnetic resonance (NMR) spectroscopy identified the reaction products as 4′-ClGem (5-(4-chloro-2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid) 4′,6′-diClGem (5-(4,6-dichloro-2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid), and 3′,4′,6′-triClGem (5-(3,4,6-trichloro-2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid), products consistent with electrophilic aromatic substitution reactions. The rapid reaction of gemfibrozil with free chlorine at pH conditions relevant to water treatment indicates that a mixture of chlorinated gemfibrozils is likely to be found in wastewater disinfected with chlorine.

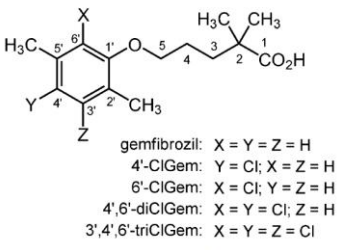

Fig. 1 – Structure of gemfibrozil and the structures deduced for the gemfibrozil-HOCl reaction products.

Experiments were performed at a pH ranging from 3 to 9 in order to determine potential reaction products formed from the reaction between gemfibrozil and free chlorine. These experiments were conducted by reacting sodium hypochlorite with 40 μg/L of gemfibrozil in Ultra Pure water in an 850:1 Molar ratio for various reaction times ranging from 0 to 60 min. Experiments were conducted in a 200 mL volume. At the end of the reaction period, blanks and samples were quenched with sodium thiosulphate in a 5:1 Molar ratio to initial chlorine concentration. Samples were processed and analyzed by GC-MS as described below for the presence of gemfibrozil as well as any potential reaction products. Based on the results of the pH trials, several different conditions were then established where each of the reaction products was produced selectively, with all other reaction products and the parent compound constituting less than 5% of total peak area on the chromatogram.

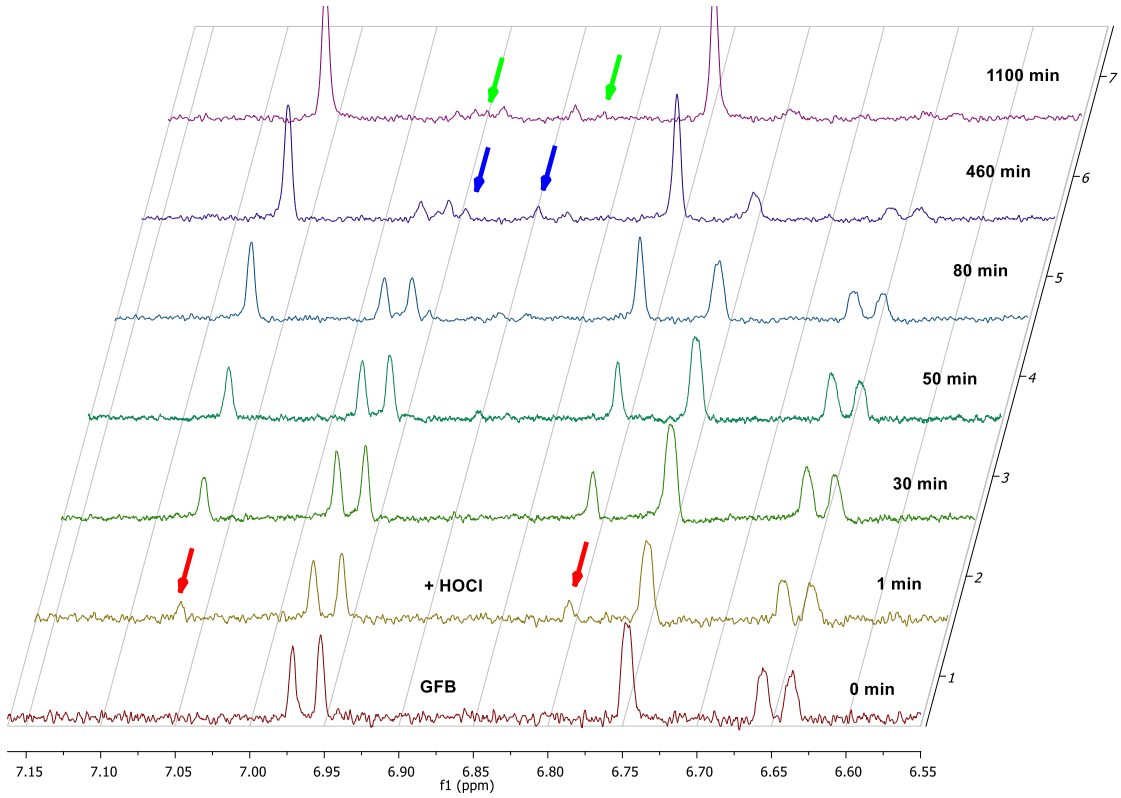

Figure 1. ^{1}H NMR spectra (aromatic region) of gemfibrozil (GFB, 2 µM) and (HOCl, 4 µM) in D_2O (pH ~ 7). *p*-chloro-gemfibrozil (red arrows), *o*-chloro-gemfibrozil (blue arrows), *m*-chloro-gemfibrozil (green arrows).

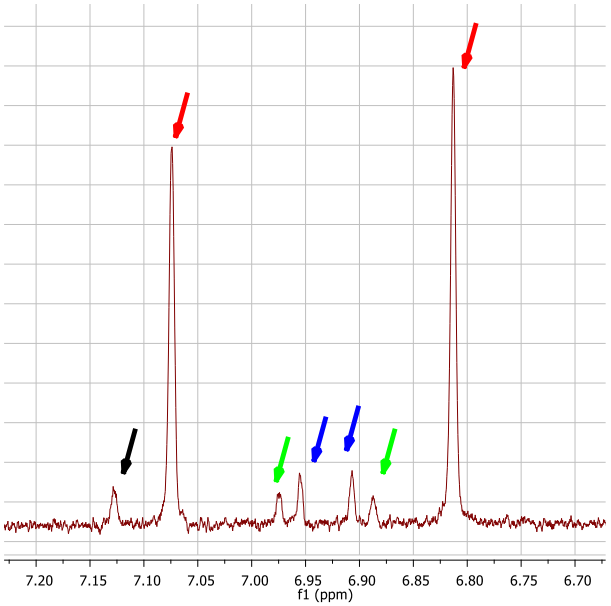

Figure 2. ^{1}H NMR spectra (aromatic region) of gemfibrozil (GFB, 2 µM) and (HOCl, 4 µM) in D_2O (pH ~ 7) taken after 1 day. *p*-chloro-gemfibrozil (red arrows), *o*-chloro-gemfibrozil (blue arrows), *m*-chloro-gemfibrozil (green arrows), dichloro-gemfibrozil (black arrow).

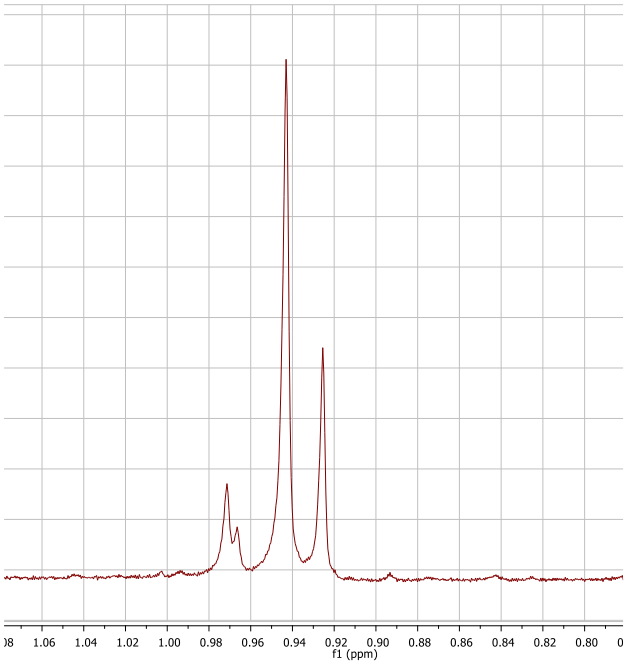

Figure 3. ^{1}H NMR spectra (upfield region) of gemfibrozil (GFB, 2 μM) and (HOCl, 4 μM) in D_2O (pH ~ 7) taken after 1 day. Four different methyl signals correspond to four chlorinated products: *p*-chloro-gemfibrozil, *o*-chloro-gemfibrozil, *m*-chloro-gemfibrozil, dichloro-gemfibrozil.

UPLC-QTOF data

Total ion chromatogram (TIC) data (Extracted reaction times)

(1) Gemfibrozil — relative TIC intensity

Time (min)	GEM (1), % of base peak
0	100
5	75
10	55
20	30
30	15

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(2) Chloro-gemfibrozil — relative TIC intensity

Time (min)	Cl-GEM (2), % of base peak
0	0
5	20
10	40
20	60
30	70

Key qualitative observation:
The decrease of GEM and the increase of Cl-GEM are complementary and monotonic, consistent with direct precursor–product formation under chlorination.

Apparent chlorination kinetics (parent decay)

Kinetic assumption
Under constant oxidant availability and low analyte concentration, gemfibrozil decay can be approximated as pseudo-first-order:

$$\ln\left(\frac{C}{C_0}\right) = -k_{\text{app}}t$$

TIC intensity is used as a proxy for concentration.

Log-transformed GEM data

Time (min)	GEM %	ln(C/C₀)
0	100	0.00
5	75	−0.29
10	55	−0.60
20	30	−1.20
30	15	−1.90

A linear fit through these points gives:

- Slope ≈ −0.064 min^{-1}

Derived kinetic parameters

Apparent chlorination rate constant

- $k_{app} \approx 6 \times 10^{-2}$ min^{-1}

Half-life

$$t_{1/2} = \frac{\ln 2}{k_{\text{app}}}$$

- $t_{1/2} \approx 11–12$ min

This is fully consistent with:

- rapid electrophilic aromatic substitution on an activated phenoxy ring,
- the near-complete conversion observed within ~30 min,
- ^{1}H NMR detection of chloro-products.

Approximate relative abundances of gemfibrozil and chloro-gemfibrozil were estimated by digitisation of normalised total ion chromatograms (TICs). Although TIC intensities are semi-quantitative and influenced by ionisation efficiency, the monotonic decrease of gemfibrozil and concomitant increase of chloro-gemfibrozil support a direct precursor–product relationship. Under these conditions, gemfibrozil decay followed apparent pseudo-first-order kinetics with an estimated rate constant of ~6×10^{-2} min^{-1} ($t_{1/2} \approx 12$ min), indicating rapid chlorination in deionised water.

Rate constants derived from TIC data should be interpreted as screening-level estimates, as TIC normalisation does not account for potential differences in ionisation efficiency between parent and transformation products.

NMR Kinetics overlay

Compound-specific kinetic evidence

- Chlorination is strongly pH-dependent. In deionized water, GEM oxidation showed no statistically significant degradation at pH > 7, while oxidation between pH 4–7 was represented by first-order kinetics in our study's model framework
- Product formation evidence supports fast ring substitution under relevant conditions

Screening-level half-lives using HOCl proxy kinetics
To provide order-of-magnitude time scales for chlorinations, the Pattison & Davies HOCl dataset as proxy bounds (widely used for HOCl reactivity classes) was used: His/amine fast; Tyr/activated aromatic moderate; backbone amides slow.[i]

Using the same pseudo-first-order approximation:

$$k_{\text{obs}} \approx k_{\text{HOCl}}[\text{HOCl}], t_{1/2} = \ln 2 / k_{\text{obs}}$$

and the HOCl/OCl^- speciation premise (pH effect on [HOCl])

Used scenario: free chlorine residual 0.5 mg/L as Cl_2; compare pH 7 vs 8.

- Ring chlorination (activated aromatic proxy; Tyr-like k ≈ 44 M^{-1} s^{-1})
  t½ on the order of tens of minutes at pH ~7, extending toward hours at pH ~8 (due to lower [HOCl]).

Photodegradation of gemfibrozil (365 nm)

Photodegradation follows pseudo-first-order kinetics under constant irradiation:

$$\ln\left(\frac{C}{C_0}\right) = -k_{\text{app}}\, t$$

Apparent rate constant (k_{app}) obtained from the slope of ln(C/C₀) vs time.

Half-life:

$$t_{1/2} = \frac{\ln 2}{k_{\text{app}}}$$

pH 7.0 — apparent photodegradation kinetics

Time (min)	ln(C/C₀)
0	0.00
15	−0.35
30	−0.75
45	−1.10
60	−1.45
75	−1.80
90	−2.10

Linear regression

Slope ≈ −0.023 min^{-1}

Derived kinetic parameters

- k_{app}(pH 7) ≈ 2.3 × 10^{-2} min^{-1}
- $t_{1/2}$(pH 7) ≈ 30 min

pH 9.0 — apparent photodegradation kinetics

Time (min)	ln(C/C₀)
0	0.00
15	−0.20
30	−0.45
45	−0.70
60	−0.95
75	−1.20
90	−1.45

Linear regression

Slope ≈ −0.016 min^{-1}

Derived kinetic parameters
k_{app}(pH 9) ≈ 1.6×10^{-2} min^{-1}
$t_{1/2}$(pH 9) ≈ 43 min

Comparative interpretation

pH	k_{app} (min^{-1})	$t_{1/2}$ (min)	Relative stability
7	~0.023	~30	lower
9	~0.016	~43	higher

Key conclusions:

- Gemfibrozil photodegrades ~1.4× more slowly at pH 9 than at pH 7 under identical irradiation.
- This trend is fully consistent with:
    - increased ionisation of the carboxylate at alkaline pH,
    - altered excited-state behaviour,
    - reduced direct photolysis efficiency.

[i] Pattison DI, Davies MJ. Absolute rate constants for the reaction of hypochlorous acid with protein side chains and peptide bonds. Chem Res Toxicol. 2001 Oct;14(10):1453-64. doi: 10.1021/tx0155451. PMID: 11599938.