

Research Articles

Transformation and Ecotoxicity of Carbamic Pesticides in Water

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DOI: <http://dx.doi.org/10.1065/espr2005.10.285>**Abstract**

Background. *N*-methylcarbamate insecticides are widely used chemicals for crop protection. This study examines the hydrolytic and photolytic cleavage of benfuracarb, carbosulfan and carbofuran under natural conditions. Their toxicity and that of the corresponding main degradation products toward aquatic organisms were evaluated.

Methods. Suspensions of benfuracarb, carbosulfan and carbofuran in water were exposed to sunlight, with one set of dark controls, for 6 days, and analyzed by ¹H-NMR and HPLC. Acute toxicity tests were performed on *Brachionus calyciflorus*, *Daphnia magna*, and *Thamnocephalus platyurus*. Chronic tests were performed on *Pseudokirchneriella subcapitata*, and *Ceriodaphnia dubia*.

Results and Discussion. Under sunlight irradiation, benfuracarb and carbosulfan gave off carbofuran and carbofuran-phenol, while only carbofuran was detected in the dark experiments. The latter was degraded to phenol by exposure to sunlight. Effects of pH, humic acid and KNO₃ were evaluated by kinetics on dilute solutions in the dark and by UV irradiation, which evidenced the lability of the pesticide at pH 9. All three pesticides and phenol exhibited acute and higher chronic toxicity towards the aquatic organisms tested.

Conclusion. Investigation on the hydrolysis and photolysis of benfuracarb and carbosulfan under natural conditions provides evidence concerning the selective decay to carbofuran and/or phenol. Carbofuran is found to be more persistent and toxic.

Recommendations and Outlook. The decay of benfuracarb and carbosulfan to carbofuran and the relative stability of this latter pesticide account for many papers that report the detection of carbofuran in water, fruits and vegetables.

Keywords: Acute and chronic bioassays; aquatic toxicity; benfuracarb; carbamic pesticides in water; carbofuran; carbosulfan; hydrolysis; photodegradation

Introduction

In recent years, the importance of non-biological (abiotic) alteration in the breakdown of pesticides has been widely noticed and has stimulated a large amount of research concerning degradation mechanisms, kinetics, isolation and toxicity of degradation products. Particular interest is focused on the toxic potential, since the analysis and monitoring of some metabolites from chemical substances have turned up

in the water framework directives of many countries of the world due to their toxicity which is sometimes even more severe than that of the parent molecule. Our objective in this study was to determine the main products of hydrolytic and photolytic cleavage of three carbamic insecticides: benfuracarb (1), carbosulfan (2) and carbofuran (3) (Fig. 1).

Alteration has been observed for carbosulfan in water/methanol (3:2 v/v) solutions [2], and for benfuracarb by photolysis in methanol [3]. Much more attention has been addressed to carbofuran, which has proven to be scarcely sensible to both hydrolysis and photolysis [4]. In particular, photodecomposition via C-O heterolysis of the carbamate group followed by ring opening has been observed in water using 254 nm UV light and has been seen to result in a substituted catechol moiety with a tert-butyl alcohol substituent and its corresponding dehydration product [5]. Moreover, several photoproducts, mainly derived from oxidation, methylation, chlorination and rearrangement have been detected by irradiation in various solvents under sunlight [6,7]. In this study, we examine the behavior of the three pesticides in MilliQ water solutions/dispersions using Pyrex tubes in the dark and under sunlight irradiation. All three pesticides exhibit absorption spectra in the same region (λ_{\max} 277–283 nm), with a tail extending to 350 nm. The effect of pH, humic acid and nitrate is also investigated. Humate and nitrate are found in aquatic environments and, sometimes, induce photodegradation [8,9].

As known, *N*-methylcarbamate insecticides are chemicals widely used for crop protection and are considered toxic for the environment and for human health, as carbamates are generally inhibitors of acetylcholinesterase [1]. The extensive pollution of the soil determines the transfer of these

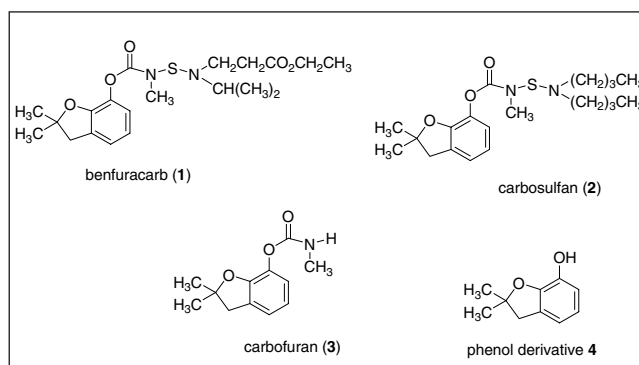


Fig. 1: Structures of pesticides and phenol derivative 4

harmful compounds into other media such as ground waters and surface waters. In this study, acute and chronic toxicity tests, incorporated into a multitrophic battery of organisms (producers and consumers of carbon) were used to measure the effects that may occur if these pesticides reach surface waters. Furthermore, to better evaluate the potential environmental risk, bioassays were enlarged to the main degradation compound.

1 Transformation of Pesticides in Water and/or Sunlight

1.1 Materials and methods

Benfuracarb (1), carbosulfan (2) and carbofuran (3) were commercially available by Aldrich-Fluka and used without further purification. Irradiations were performed by exposure of the compounds to sunlight or to a 500 W high-pressure mercury lamp (Helios Italquartz). In a standard procedure, suspensions of benfuracarb (205 ppm) and carbosulfan (190 ppm) in MilliQ water were exposed to sunlight in Pyrex flasks, under aerobic conditions. Each experiment was performed in duplicate, with one set of dark controls. After 6 days, each reaction mixture was evaporated in a vacuum and residues were analyzed by ¹H-NMR on a 500 MHz spectrometer (Varian INOVA) and by HPLC (Agilent 1100 HPLC system equipped with UV detector). Control experiments showed that more diluted solutions afforded similar results.

Experiments using the same concentrations of pesticides were carried out at pH 5.0 and 9.0 using NaH₂PO₄/Na₂HPO₄, by adjusting the pH with HCl 0.2 M and KOH 0.2 M. After 6 days, each reaction mixture was neutralized and analyzed by ¹H-NMR and HPLC [column Spherex 10 μm OH (DIOL), eluent hexane/ethyl acetate (4:1), λ = 280 nm].

Experiments were also performed in the presence of KNO₃ (10 mg/L) and with humic acid (5 mg/L). After 6 days, each

reaction mixture was evaporated in a vacuum and analyzed by ¹H-NMR and HPLC.

Carbofuran (110 ppm) in MilliQ water was treated according to the standard procedure. After six days, it was recovered unchanged in the dark while it decomposed to about 7% under irradiation, leading only to phenol derivative 4 (¹H NMR and HPLC).

Kinetic experiments of carbofuran (4 ppm) in MilliQ water were performed in Pyrex tubes and this compound was irradiated with a UV lamp. At selected time intervals, samples were collected and analyzed directly using HPLC [column Synergy 4 μm MAX-RP80A, eluent water/methanol/acetonitrile (21:14:15), λ = 254 nm].

Carbofuran-phenol (4) (Fig. 1) was isolated from irradiation experiments by repeated TLC [hexane/ethyl acetate (7:3)], and identified by comparison of ¹H and ¹³C-NMR data with those of an authentic sample which was obtained by treating carbofuran (0.09 M) with methanolic KOH (5%): ¹H-NMR (CDCl₃): δ 6.72 (m, 3H), 3.04 (s, 2H), 1.50 (s, 6H). ¹³C-NMR (CDCl₃): δ 145.8 (C-2), 140.3 (C-1), 127.8 (C-3), 120.7 (C-4, C-5), 117.0 (C-6), 88.0 (C-7), 43.5 (C-8), 28.2 (C-9, C-10).

1.2 Results

Benfuracarb was unstable in water, decomposing to carbofuran either in the dark or in irradiating conditions (Table 1).

Under sunlight, a small amount of phenol derivative 4 was also identified. The pesticide was not sensible to small pH variations or to the presence of nitrate or humic acid, thus giving the same products in comparable amounts. Carbosulfan was seen to be more stable under all conditions examined, except in the presence of humic acid, which led to carbofuran and/or phenol at about 40% degradation, either

Table 1: Hydrolysis /photolysis of benfuracarb (1) and carbosulfan (2) under different conditions

Condition ^a	Starting pesticide (%) ^b		Degradation products (%) ^b	
	Dark/Sunlight		Dark/Sunlight	
	Benfuracarb (1)		Carbofuran (3)	Phenol (4)
H ₂ O	79/57		14/21	1/10
pH 5.1	63/55		26/31	-/2
pH 9.0	85/74		6/13	1/6
KNO ₃ ^c	81/70		13/18	-/3
Humic acid ^d	80/82		11/18	-/2
	Carbosulfan (2)			
H ₂ O	93/87		4/5	-/3
pH 5.1	87/82		7/7	-/3
pH 9.0	85/80		11/6	-/4
KNO ₃ ^c	91/83		5/6	-/8
Humic acid ^d	58/55		27/26	2/7

^a Dispersion of the pesticide in MilliQ water (205 mg/L for 1; 190 mg/L for 2); r.t.; Pyrex tube

^b Deduced by ¹H NMR and HPLC

^c 10 mg/L

^d 5 mg/L

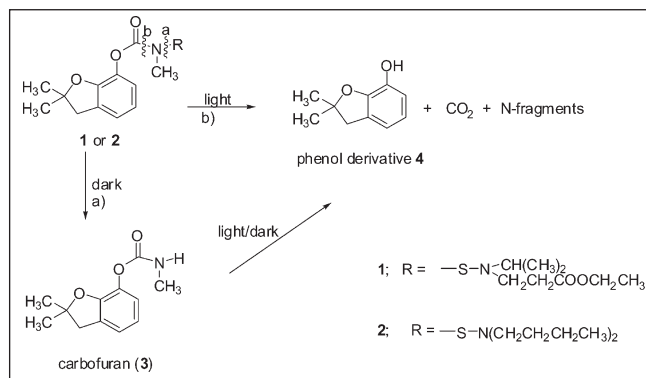


Fig. 2: Proposed degradation pathways of carbamic pesticides in water

in the dark or under sunlight (Table 1). These results showed that the cleavage of the weak S-N bond was the main process observed in the dark (Fig. 2). According to previous data reported in the literature [2], the finding of only carbofuran provides evidence for the greater lability of the carbamate nitrogen sulfur bond as compared with the amino nitrogen-sulfur bond. Under sunlight, the photocleavage of the light-sensitive [10] carbamate bond also occurs, even to a little extent leading to phenol derivative 4. As reported for photolysis of carbamates in water [11,12], the process should occur with initial homolytic cleavage of the phenoxy bond to afford the fragments which liberate the phenol derivative 4, CO₂ and nitrogen-containing fragments (Fig. 2).

Both hydrolysis and photochemical processes do not appear to be affected by variation of pH or by the presence of additives such as humic acid or KNO₃. A slight enhancement of phenol derivative 4 was only observed at pH 9, due to the fact that carbamate is susceptible to hydrolysis particularly in basic media. The enhanced degradation of carbofuran in the presence of humic acid should not be due to acidic or sensitizing effects, but rather to adsorption phenomena which might make the pesticide more susceptible to hydrolysis. The role of suspended sediment or soil on the persistence of pesticides has been observed and appeared not to be strictly related to the chemical structure [13].

The presence of only carbamic function makes carbofuran more persistent, and no appreciable degradation is to be observed in the dark after six days. Within the same time period, the degradation rate was barely enhanced by irra-

diation [14,15] and led to the production of only about 7% phenol derivative. The kinetics data in different conditions, using UV lamp, clearly provide evidence for the enhanced degradation induced by light (Table 2). The photolysis with this lamp is faster than that under sunlight, probably due to the higher UV lamp intensities compared to the natural light. The results at pH 9 are significant, in fact the basic medium alone or with light contributes to promote the C-O bond cleavage. Experiments performed flushing the solution with N₂ showed that phototransformation of carbofuran to phenol does not require aerobic conditions (data not shown).

2 Toxicity of the Three Pesticides and Phenol

The three pesticides and their main derivative 4 were subjected to toxicological investigations to evaluate their acute, as well as the subtle, multigenerational effects on producers and primary consumers of the aquatic chain. Acute toxicity tests were performed on the rotifer *Brachionus calyciflorus* and two crustaceans, *Daphnia magna* and *Thamnocephalus platyurus*, while chronic tests comprised a producer, the alga *Pseudokirchneriella subcapitata* and a consumer, the crustacean *Ceriodaphnia dubia*.

2.1 Procedure

Benfuracarb, carbofuran and phenol derivative 4 were initially dissolved in dimethylsulfoxide and then diluted in double deionized water. The DMSO concentration was kept constant at 0.01% (v/v). A solvent only control was included in each experiment to detect the possible effect of the vehicle. At the same time as acute and chronic toxicity tests, reference assays were performed with potassium dichromate (Aldrich Chemical, St. Louis, MO, USA) for all the organisms except *C. dubia*, for which pentahydrate copper sulphate (Aldrich) was used.

The acute tests were performed in accordance with the standard protocols for *B. calyciflorus* and *D. magna* [16,17], and following the operational procedure provided by MicroBioTest, Nazareth, Belgium for *T. platyurus*. Neonates aged less than 24h were exposed to each concentration of pesticides in static tests.

Table 2: Kinetics of carbofuran (3)

Condition ^a	UV ^b		Dark	
	k(h ⁻¹)	t _{1/2} (h)	k(h ⁻¹)	t _{1/2} (h)
pH 5.0	1.9 × 10 ⁻³	365	1.0 × 10 ⁻⁴	6931
pH 7.1	1.4 × 10 ⁻³	495	9.0 × 10 ⁻⁵	7701
pH 9.0	0.67	1.0	0.21	3.0
MilliQ water	1.0 × 10 ⁻³	693	1.0 × 10 ⁻⁴	6931
Humic acid ^c	8.0 × 10 ⁻⁴	866	9.0 × 10 ⁻⁵	7701
Nitrate ^d	8.0 × 10 ⁻⁴	866	9.5 × 10 ⁻⁵	7296

^a Solution of the pesticide (4 mg/L) in milliQ water; r.t.; Pyrex tube

^b 500W high-pressure mercury lamp (Pyrex filter)

^c 5 mg/L

^d 10 mg/L

Table 3: Acute toxicity tests L(E)C50 in mg/L with a 95% confidence range

Compound	<i>Brachionus calyciflorus</i>	<i>Thamnocephalus platyurus</i>	<i>Daphnia magna</i>
1	48% mortality at 200 mg/L	3.66 (2.27–5.29)	0.13 (0.11–0.15)
2	95.7 (85.5–103.4)	8.93 (6.02–13.26)	0.004 (0.003–0.006)
3	14.1 (13.3–14.9)	2.32 (1.53–3.51)	0.01 (0.01–0.02)
4	55.2 (41.6–73.2)	111 (102–122)	18.8 (10.4–38.0)

Table 4: Chronic toxicity tests L(E)C50 in mg/L with a 95% confidence range

Compound	<i>Ceriodaphnia dubia</i>	<i>Pseudokirchneriella subcapitata</i>
1	1.0×10^{-6}	14.1 (10.4–26.0)
2	8.2×10^{-8} (5.4×10^{-8} – 1.8×10^{-7})	4.6 (3.4–6.2)
3	1.8×10^{-8} (1.2×10^{-8} – 2.6×10^{-8})	2.6 (2.2–3.2)
4	4.7×10^{-5} (3.5×10^{-5} – 7.0×10^{-5})	11.4 (10.9–18.7)

The reproductive capability was assessed in a semi-static test according to the standard protocol for *C. dubia* [18], while the algal growth inhibition was evaluated in a static test according to procedure 8692 [19].

Statistic treatment of data. All results, except algal tests, were analyzed using the Toxcalc™ program [20]. For acute toxicity tests, the LC₅₀ and EC₅₀ with 95% confidence intervals were calculated by concentration/response regression using the probit or trimmed Spearman-Kärber method, as appropriate. For the test with *C. dubia*, the value of EC₅₀ with 95% confidence intervals was calculated using the Maximum Likelihood-Logit method. From raw test data of algae, the growth inhibition in percentage was calculated by integrating the mean values from t₀ to t₇₂ h (area under the curve). Inhibition (%) values were tabulated against log-transformed data of concentrations to evaluate the test concentration corresponding to 50% algal growth inhibition.

2.2 Toxicity characterization

Acute and chronic toxicity data, expressed as median effective concentrations (LC₅₀ and EC₅₀ in mg/L), are reported in Tables 3 and 4, respectively.

For all the compounds, the acute effects were found for concentrations ranging from 2.32 mg/L (carbofuran versus *T. platyurus*) to 48% mortality at 200 mg/L (benfuracarb versus *B. calyciflorus*), thus suggesting the limited acute ecotoxicity of these compounds. As an exception, *D. magna* was found to be more sensitive particularly to parent molecules. Chronic results demonstrated that all the tested pesticides had a strong toxic potential for the crustacean *C. dubia* with EC₅₀ values which were at least two orders of magnitude below the acute toxic level and five orders below the chronic level for the algae. Among the three investigated pesticides, carbosulfan and carbofuran were the most active and the phenol derivative 4 was generally less toxic than the parent compounds.

By the comparison of data, it was found that the various species utilized were not in the same order of sensitivity, thus suggesting that the investigated pesticides and the phenolic product 4 showed a different toxic impact on non-target organisms.

3 Conclusion

This work reports the first data on the hydrolytic and photolytic stability of benfuracarb, carbosulfan and carbofuran under natural conditions and provides evidence concerning the selective decay of the first two pesticides to carbofuran and/or phenol derivative 4. The S-N bond breakage occurs easily under all the conditions used, while carbamic bond cleavage is favored under light and in basic media. Accordingly, phenol is hardly formed by carbofuran and becomes appreciable at pH 9 and/or by irradiation. The high persistence of carbofuran accounts for many papers that have reported the detection of this pesticide in water, fruits and vegetables [18]. These data highlight the environmental risk of carbofuran that in our results was found to be the most toxic towards all the exposed organisms. It is likely that the different results observed under the above conditions, with respect to that reported in previous work, are probably due to the different reaction conditions (the absence of organic solvents, even if in small amount, which might favor association processes, [2,3,6] or the use of more diluted solution [7] or the use of natural sunlight [5]). It is interesting to note that phenol derivative 4 has also been found as an enzymatic degradation product from soil microorganisms [22]. This finding may be assumed important, especially for chronic exposure of aquatic organisms to carbamic pesticides, because of their effective concentrations which are found to be active for *C. dubia*.

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Sensitized Photooxygenation of the Fungicide Furalaxyl

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Background. The photolysis of pesticides is of high current interest since light is one of the most important abiotic factors which are responsible for the environmental fate of these substances and may induce their conversion into noxious products. The action of light can also be mediated by oxygen and synthetic or naturally occurring substances which act as sensitizers. Our objective in this study was to investigate the photochemical behaviour of the systemic fungicide furalaxyl in the presence of oxygen and various sensitizers, and to compare the toxicity of the main photoproduct(s) to that of the parent compound. Previous reports on the direct photolysis of the pesticide demonstrated a very slow degradation and the only identified photoproducts were N-2,6-xylyl-D,L-alaninate and 2,6-dimethylaniline.

Methods. Solutions of furalaxyl in CH₃CN were photooxygenated using a 500W high-pressure mercury lamp (through a Pyrex glass filter, λ>300 nm) or a 650W halogen lamp or sunlight and the proper sensitizer. When sunlight was used, aqueous solutions were employed. The photodegradation was checked by NMR and/or GC-MS. The photoproducts were spectroscopically evidenced and, when possible, isolated chromatographically. Acute toxicity tests were performed on the rotifer *Brachionus calyciflorus*, the crustacean cladoceran *Daphnia magna* and the anostracan *Thamnocephalus platyurus*, while chronic toxicity tests (sublethal endpoints) comprised a producer, the alga *Pseudokirchneriella subcapitata* and the crustacean *Ceriodaphnia dubia*, as a consumer.

Results and Discussion. In the presence of both oxygen and sensitizer, furalaxyl underwent rapid photochemical transformations mainly to N-disubstituted formamide, maleic anhydride and a 2(5H)-furanone derivative. The formation of these products was rationalized in terms of a furan endoperoxide intermediate derived from the reaction of furalaxyl with active dioxygenated species (singlet oxygen, superoxide anion or ground state oxygen). The 2(5H)-furanone exhibited a higher toxicity than the parent compound.

Conclusion. This work reports the first data on the photosensitized oxygenation of furalaxyl with evidence of the high tendency of the pesticide to undergo photodegradation under these conditions leading, among other things, to a 2(5H)-furanone, which is more toxic than the starting furalaxyl towards aquatic organisms.

Recommendations and Outlook. Investigation highlights that the photolytic fate of a pesticide, although quite stable to direct photoreaction due to its low absorption of solar radiation at ground level, can be significantly influenced in the environment by the presence of substances with energy or electron-transfer properties as natural dyes, e.g. chlorophyll, or synthetic pollutants, e.g. polycyclic aromatic hydrocarbons (PAH).

Keywords: Furalaxyl; 2(5H)-furanone; pesticides; photodegradation; sensitized photooxygenation; toxicity towards aquatic organisms