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Photochemistry of Imidacloprid in Model Systems

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The photochemical behavior of the neonicotinoid insecticide imidacloprid was studied with regard to different chemical environments. Different model solvents simulated the structure moieties mainly occurring in waxes and cutin of the plant cuticle. Cyclohexane and cyclohexene substituted saturated and unsaturated hydrocarbon chains, whereas ethanol and 2-propanol were models for primary and secondary alcohol groups of cuticular components. After 5 h of irradiation, imidacloprid was completely degraded in all solvents. With 88−96 mol% 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine was formed as the main product, whereas 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one was identified as minor product in the range 4−6 mol%. By contrast, besides the photoproducts formed in organic solvents, irradiation of the solid imidacloprid on a glass surface delivered a complex variety of unidentified photoproducts. The nucleophilic addition reaction of the main photoproduct, 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine, with both cyclohexene oxide and methyl 9,10-epoxystearate as model compounds indicates that epoxidized cutin acids are possible reaction partners for the formation of plant cuticle bound residues of imidacloprid, which could explain the reported findings of nonextractable residues of imidacloprid in plants.

KEYWORDS: Imidacloprid; photodegradation; bound residues

INTRODUCTION

The neonicotinoids, with imidacloprid (1-[(6-chloropyridin-3-yl)methyl]-*N*-nitroimidazolidin-2-imine, **1**, **Scheme 1**) as the most prominent representative, are a rather new class of insecticides, which differ in their mode of action from earlier pesticides like organophosphates, carbamates, organochlorines, or pyrethroids. They target at the nicotinic acetylcholine receptor, acting as agonists of this receptor (*1*). Because of high efficacy against sucking and some biting insects, systemic properties, and low mammalian toxicity, **1** is widely used in plant protection and veterinary medicine (*2*).

Photolysis of imidacloprid in water was studied by different groups (*3*−*7*), which resulted in rapid degradation and a variety of reaction products (**Scheme 1**). All groups identified 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one (**2**) as the main photoproduct besides 6-chloronicotinic acid (**3**) and 6-chloronicotinyl-aldehyde (**4**).

After spray application, there are different possible degradation pathways on plant surfaces such as hydrolysis, oxidation, or photolysis. Photoinduced reactions with constituents of the plant surface, the cuticle, resulting in the formation of bound residues, are also possible (*8*). In the 2002 evaluations of the Joint FAO/WHO Meeting on Pesticide Residues (*9*), nonextractable residues in tomatoes, with a range 0.2−0.8%, were reported after spray application of imidacloprid to tomato fruits on tomato plants. But, the formation pathway of these residues is still unknown. Scholz and Reinhard (*10*) performed photolysis studies of imidacloprid on leaves of tomato plants and described the cyclic guanidine (**5**), the cyclic urea (**2**), the nitroso (**6**, **Scheme 2**), and a hydroxy derivative as main products and up to 4.4% of nonextractable residues.

In this study, we examined the photoreactivity of **1** in different model solvents, which were introduced by Schwack et al. (*11*−*13*). Waxes and cutin as constituents of the plant cuticle provide a wide range of possible reaction partners for applied pesticides or their photoproducts. Cutin is a completely insoluble polyester consisting of interesterified saturated, unsaturated, and epoxydized hydroxy fatty acids, which is associated by intra- and epicuticular lipids called waxes extractable by organic solvents (*14*). The models cyclohexane and cyclohexene substitute saturated and unsaturated hydrocarbons as they occur (e.g., as alkanes and alkenes, fatty acids, triterpenes, and sterols) in the plant cuticle. Cutin acids, free fatty alcohols, or sterols contain primary and secondary alcohol groups, whose influence was studied by irradiations in ethanol and 2-propanol. In former studies, most pesticides showed different degradation rates and different product spectra depending on the solvent used (*11*−*13*). Among the products, photoaddition products with alcohols and olefins were detected that deliver reaction mechanisms for the formation of covalently bound residues with constituents of the plant cuticle. As imidacloprid, parathion also contains a nitro group, which was first photoreduced to nitrosoparathion and finally to aminoparathion, both of which are rather reactive toward plant cuticle constituents (*15*−*17*). Thus, we conducted photolysis studies using our well-established model systems to

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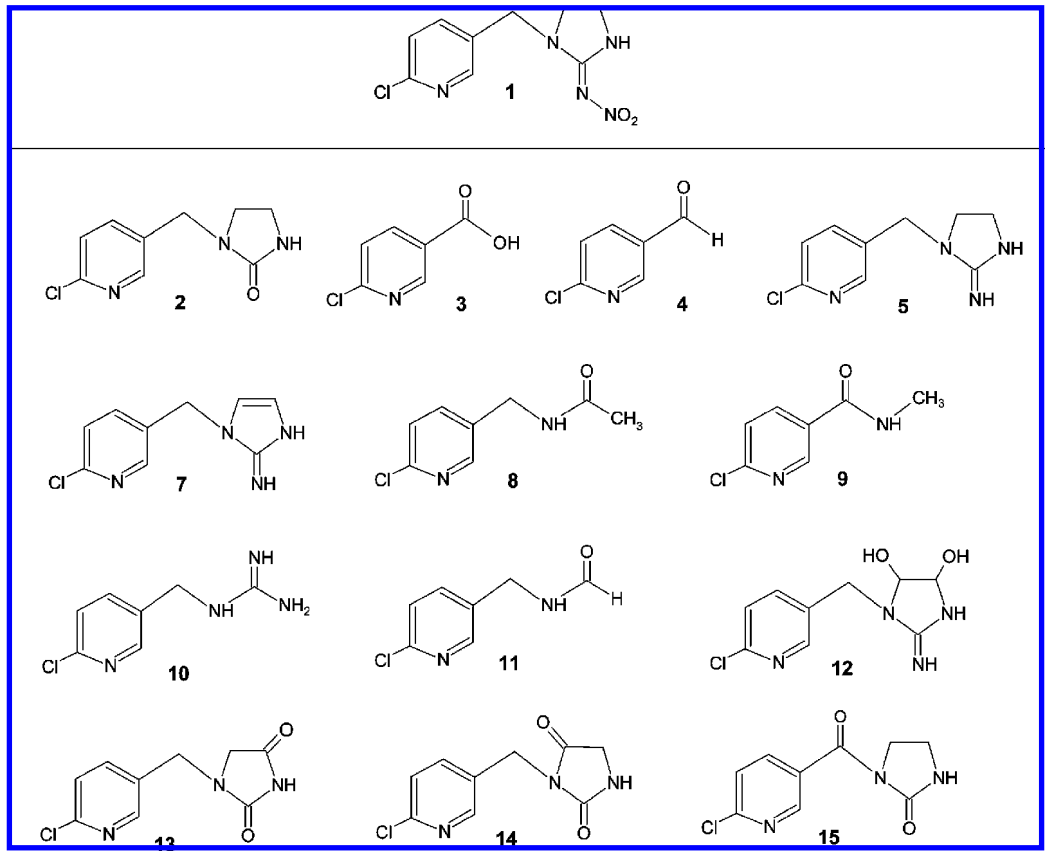
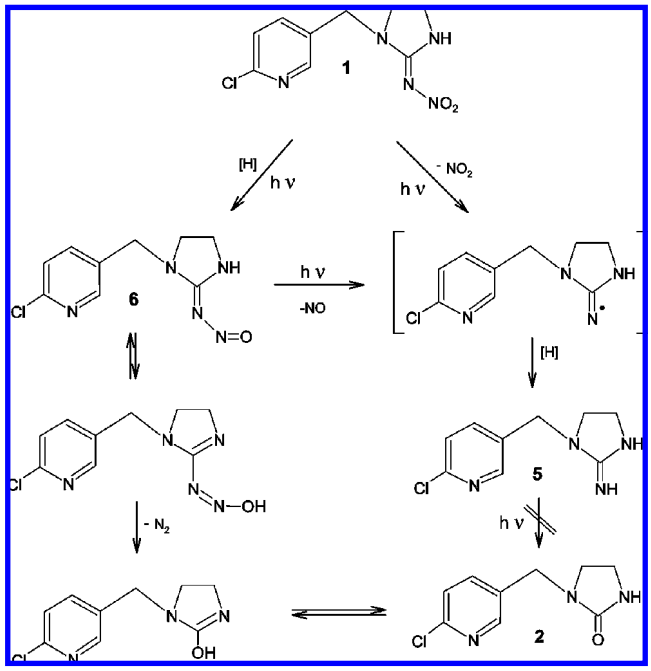
Scheme 1. Structural Formula of Imidacloprid (**1**) and Its Photoproducts in Water Described in the Literature (*3*−*7*)



Scheme 2. Proposed Mechanism of the Photolysis of Imidacloprid (**1**) Dissolved in Organic Solvents



study and understand the general photochemistry of imidacloprid to be expected on fruit and leaf surfaces.

MATERIALS AND METHODS

Reagents. All solvents used were of analytical grade (Merck, Darmstadt, Germany) and distilled before use. Cyclohexene was distilled over P_2O_5 (Roth, Karlsruhe, Germany). Cyclohexene oxide was purchased from Fluka (Buchs, Switzerland). Water was purified by a Synergy ultrapure water system (Millipore GmbH, Schwalbach, Germany).

Imidacloprid was precipitated from Confidor (200 g/L, Bayer, Leverkusen, Germany) by addition of water. After filtration and washing with water, the crude product was recrystallized twice from methanol yielding colorless crystals with a melting point of 144 °C (143−144 °C (*18*)). The determined log ε at 269 nm (methanol) of 4.12 matched the respective value of the commercial standard substance (99.9%, Pestanal, Riedel de Haen, Seelze, Germany). LC/MS (ESI+): *m*/*z* 294 ($[M + K]^+$, 4%), 278 ($[M + Na]^+$, 5%), 256 ($[M + H]^+$, 100%), 210 ($[M + H - NO_2]^{+\bullet}$, 23%), 209 ($[M + H - HNO_2]^+$, 18%), 175 ($[M + H - NO_2 - Cl]^+$, 14%).

General techniques. Thin-layer chromatography (TLC) was performed on silica gel 60 TLC aluminum sheets (20 cm × 20 cm, F_{254}, Merck, Darmstadt, Germany) with ethyl acetate/methanol/toluene/acetic acid (98%) (80/60/20/1) as mobile phase.

High-performance liquid chromatography (HPLC) was carried out on a HP 1100 system (Agilent, Waldbronn, Germany) equipped with a degasser, an autosampler, a quarternary pump, and a diode array detector (DAD). For separation at 25 °C, a reversed-phase column Eurospher 100-C18 (5 μm, 250 mm × 3 mm, Knauer, Berlin, Germany) with a guard column (C_{18}, 5 μm, 5 mm × 3 mm) and a gradient system with methanol/phosphate buffer (20 mM, pH 4.0, 2% methanol) was used. The methanol content of the gradient at a flow rate of 0.5 mL/min was as follows: 0 min, 5%; 0−2 min, 5−25%; 2−7 min, 25−40%; 7−13 min, 40−50%; 13−18 min, 50%; 18−20 min, 50−100%; 20−30 min, 100%; 30−35 min, 100−5%. An aliquot of 10 μL was injected. DAD detection was performed at screening wavelengths of 235, 270, and 320 nm; for quantification, 270 nm was used for all compounds. Data acquisition and processing were carried out with HP ChemStation software (rev. A.06.01).

The high-performance liquid chromatography−mass spectrometry (LC/MS) system consisted of an HPLC system HP 1100 as described above and a VG platform II quadrupole mass spectrometer (Micromass, Manchester, U.K.) equipped with an electrospray interface (ESI). Apart from the solvents (ammonium formate buffer (10 mM, pH 4.0) and

methanol) used for LC separation, all LC parameters remained the same as for HPLC/DAD analysis. MS parameters were as follows: ESI+; source temperature, 120 °C; capillary 3.5 kV; HV lens 0.5 kV; cone ramp 20−60 V. MS was operated in full-scan mode (*m/z* 80−1200). For data acquisition and processing, MassLynx 3.2 software was employed.

An LCT Premier XE LC/TOF-MS system (Waters, Manchester, U.K.) was used for the determination of accurate masses.

Preparative HPLC was carried out on a system consisting of two pumps (Kronlab, Sinsheim, Germany), an A 0293 variable-wavelength monitor (Knauer, Berlin, Germany), a C-R3A integrator (Shimadzu, Duisburg, Germany), and a YMC HPLC column (guard column 50 mm × 20 mm; column, 250 mm × 20 mm; YMC-Pack ODS-A, 10 μm); a gradient system consisting of ammonium formate buffer (10 mM, pH 4.0) and methanol as eluents (flow rate = 20 mL/min), with gradient as above, was used. Pump flow and gradient were controlled by Prepcon software (SCPA GmbH, Weyhe-Leeste, Germany).

High-speed countercurrent chromatography (HSCCC) was performed with a model CCC-1000 (Pharma-Tech Research Corp., Baltimore, Maryland) connected to a A 0293 variable wavelength monitor (detection at 270 nm, Knauer, Berlin, Germany) and a multirange BD 8 recorder (Kipp & Zonen, Delft, Netherlands). The two-phase solvent system consisted of water/ethyl acetate/*n*-hexane/1-butanol (10/8/1/0.5; v/v/v/v) and was pumped into the column with a Knauer HPLC pump 64 equipped with a preparative pump head. The lower phase was used as mobile phase and was introduced through the head toward the tail with a flow rate of 1.5 mL/min. The revolution speed was set at 1000 rpm. Fractions of 5 mL were collected with a Retriever 500 (ISCO, Lincoln, Nebraska).

Ultraviolet spectra were measured from 190 to 400 nm with a Cary 1E spectrophotometer (Varian, Darmstadt, Germany) using quartz glass cuvettes; scan rate: 60 nm/min; ave time: 0.1 s; data intervall: 0.1 nm.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Unity Inova 300 spectrometer (Varian, Darmstadt, Germany) at 298 K at 300 and 75 MHz (nominal frequency), respectively, in dimethyl-d_6-sulfoxide (d_6-DMSO) or d_4-methanol. Chemical shifts are given in δ (ppm) relative to trimethylsilane (TMS).

An infrared attenuated total reflectance (IR-ATR) spectrum was recorded on a Fourier transform infrared (FT-IR) spectrometer Nicolet Avatar 320 ESP (Thermo Electron, Dreieich, Germany).

Melting points were determined on a digital melting point apparatus model 8100 (Electrothermal, Southend-on-Sea, U.K.) and are not corrected.

For irradiation experiments in solutions, a metal halogen lamp (SOL 500, Dr. K. Hönle GmbH, Martinsried, Germany; 120 000 lx, 900 W/m^2) with a glass filter WG 295 (Schott Glaswerke, Mainz, Germany, λ > 280 nm) was used. Irradiation on glass surfaces was performed under a Suntest CPS + (Heraeus-Industrietechnik, Kleinostheim, Germany) with a xenon lamp, UV filters (λ > 290 nm) and air cooling; standard black temperature: 35 °C; irradiance: 250 W/m^2.

Syntheses. Synthesis of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) was performed following a modified procedure of Hayakawa et al. (*19*). In a 100 mL screw-capped bottle, 400 mg of imidacloprid (1.56 mmol) and 800 mg of stannous(II) chloride dihydrate (3.54 mmol, analytical grade, Merck, Darmstadt, Germany) in 75 mL of formic acid (98−100%, analytical grade, Merck, Darmstadt) were heated at 100 °C for 4 h. After cooling, the reaction batch was transferred into a 250 mL round-bottomed flask; 20 g of silica gel 60 (0.063−0.200 nm, for column chromatography, Merck, Darmstadt) was added, and formic acid was distilled off on a rotary evaporator under reduced pressure (40 mbar, 40 °C). The residue was suspended in 40 mL of ethyl acetate and transferred into a glass column (3 cm × 40 cm); the product was eluted with ethyl acetate/methanol (1/1, v/v). Fractions of 10 mL were analyzed by TLC, and the ones containing **5** (R_f value = 0.05) were combined. The residue obtained after evaporation was recrystallized from ethanol to yield 180 mg of colorless crystals (55% of theory; melting point 250 °C under decomposition). LC/MS (ESI+): *m/z* 211 ([M + H]$^+$). ^{1}H NMR (d_6-DMSO): δ (ppm) = 8.39 (m, 1H), 7.81 (m, 1H), 7.56 (m, 1H), 4.56 (s, 2H), 3.34−3.53 (m, 4H). ^{13}C NMR (DMSO-d_6): δ (ppm) = 160.3, 150.5, 150.0, 140.1, 131.6, 125.1, 47.7, 45.0, 41.1. IR (ATR, cm^{-1}) 3347 (w), 3130 (sh), 3037 (br, s), 2900 (m), 1663 (s), 1618 (m), 1564 (s), 1452 (m), 1383 (m), 1358 (m), 1306 (w), 1285 (m), 1244 (m), 1209 (m), 1139 (m), 1110 (s), 1097 (s), 1025 (m), 966 (w), 931 (w), 848 (m), 804 (m), 738 (s), 697 (m), 674 (s). UV (methanol): λ_{max} = 207 (log ε = 4.30), 268 nm (log ε = 3.45).

1-[(6-Chloropyridin-3-yl)methyl]imidazolidin-2-one (**2**) was synthesized by reaction of 200 mg (0.78 mmol) of imidacloprid with 20 mL of an aqueous potassium hydroxide solution (40 g/L) for 1 h at 100 °C according to Rouchaud et al. (*20*) (yield: 58.6 mg, 35% of theory).

For the preparation of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one oxohydrazone (**6**), 100 mg (0.39 mmol) of imidacloprid was reduced with 150 mg of iron powder (pure, Sigma-Aldrich, Munich, Germany) in 50 mL of an aqueous ammonium chloride solution (11 mM). After stirring for 2 h at room temperature, the reaction batch was filtered, and water was evaporated at reduced pressure (30 mbar, 40 °C). The residue was purified by HSCCC. The fraction of 185−225 mL was collected, the organic solvents were evaporated in vacuo, and the remaining aqueous solution was lyophilized to yield 28 mg of yellow crystals (30% of theory; melting point 146 °C under decomposition). LC/MS (ESI+): *m/z* 262 ([M + Na]$^+$, 10%), 278 ([M + K]$^+$, 6%), 240 ([M + H]$^+$, 100%), 210 ([M + H − NO]$^{+\bullet}$, 45%), 209 ([M + H − HNO]$^+$, 12%), 175 ([M + H − NO − Cl]$^+$, 6%). UV (methanol): λ_{max} = 212 (log ε = 3.99), 269 nm (log ε = 4.28).

Methyl 9,10-epoxystearate was synthesized by the reaction of 1.0 g (3.2 mmol) of methyl oleate (Sigma-Aldrich, Munich, Germany) with 3 mL of peracetic acid (32% in dilute acetic acid, Sigma-Aldrich) in 5 mL of diethyl ether for 20 h at room temperature. The reaction batch was poured in 20 mL of a saturated potassium carbonate solution and extracted three times with 10 mL of diethyl ether. The organic extract was dried over anhydrous sodium sulfate (Merck, Darmstadt, Germany), and the solvent was evaporated resulting in 532 mg of a colorless oil (50,4% of theory).

For kinetic studies, a solution of 10 mg (0.048 mmol) of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) and 500 μL (4.95 mmol) of cyclohexene oxide in 2 mL of methanol was stirred at ambient temperature in the dark for 14 days. Samples of 10 μL were taken, diluted with 990 μL of methanol, and analyzed by HPLC. In a preparative scale, 101 mg (0.48 mmol) of **5** was dissolved in 20 mL of methanol, 5 mL (49.5 mmol) of cyclohexene oxide were added, and the reaction batch was stirred at 50 °C for 3 days. The mixture was concentrated under vacuum to 5 mL, and 5 mL of ammonium formate buffer (10 mM, pH 4.0) was added. Separation by preparative HPLC yielded two fractions at 9.0 min (**16**, 78 mg, 52.8% of theory) and 10.5 min (**17**, 5.6 mg, 3.8% of theory).

2-({1-[(6-Chloropyridin-3-yl)methyl]imidazolidin-2-ylidene}amino)-cyclohexanol (**16**); LC/MS (ESI+): *m/z* 309 [M + H]$^+$; accurate mass calculated for $C_{15}H_{22}N_4OCl$ ([M + H]$^+$) 309.1482, found 309.1472. ^{1}H NMR (d_4-methanol): δ (ppm) = 8.39 (m, 2H), 7.84 (m, 2H), 7.54 (m, 2H), 4.74−4.60 (m, 4H), 3.77−3.55 (m, 8H), 3.55−3.43 (m, 2H), 3.32−3.21 (m, 2H), 2.16−1.90 (m, 4H), 1.90−1.74 (m, 4H), 1.32−1.24 (m, 8H). ^{13}C NMR (d_4-methanol): δ (ppm) = 158.9, 158.1, 151.0, 148.9, 148.9, 139.2, 139.2, 130.6, 130.5, 124.8, 75.0, 73.0, 69.1, 60.2, 60.0, 47.8, 45.8, 45.7, 45.2, 41.0, 34.4, 33.0, 31.4, 28.2, 24.6, 24.5, 24.2, 24.1, 24.0. UV (methanol/phosphate buffer, from DAD spectra): λ_{max} = 214, 269 nm.

2-({1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene}amino)-cyclohexanone (**17**); LC/MS (ESI+): *m/z* 307 [M + H]$^+$; accurate mass calculated for $C_{15}H_{20}N_4OCl$ ([M + H]$^+$) 307.1326, found 307.1321. ^{1}H NMR (d_4-methanol): δ (ppm) = 8.36 (m, 1H), 7.80 (m, 1H), 7.52 (m, 1H), 4.67−4.56 (m, 2H), 3.75−3.56 (m, 4H), 3.89 (m, 1H), 2.08−1.95 (m, 4H), 1.88−1.78 (m, 2H), 1.61−1.42 (m, 2H); ^{13}C NMR (d_4-methanol): δ (ppm) = 155.6, 151.2, 149.0, 139.3, 130.4, 124.8, 81.4, 51.3, 46.6, 44.6, 45.2, 39.0, 36.6, 27.0, 18.3. UV (methanol/phosphate buffer, from DAD spectra): λ_{max} = 213, 269 nm.

Methyl-9,10-epoxystearate (105 mg, 0.336 mmol) and **5** (3 mg, 0.014 mmol) were dissolved in 5 mL of methanol and heated under reflux for 24 h. The reaction mixture (10 μL) was directly injected into the LC/MS system.

Irradiation experiments. Imidacloprid (15 mg, 0.058 mmol) were dissolved in 50 mL of methanol, ethanol, 2-propanol, cyclohexane/ethanol, or cyclohexene/ethanol (20 vol% ethanol to improve solubility).

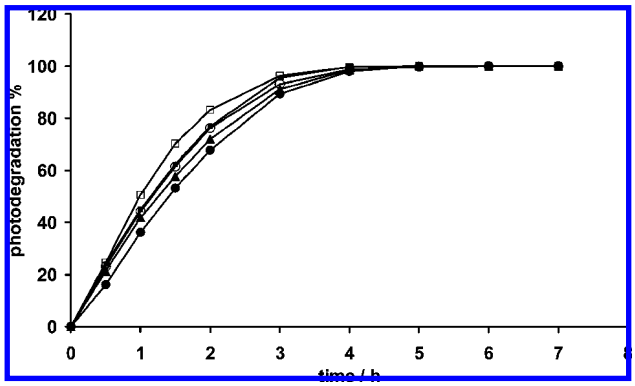


Figure 1. Photodegradation of imidacloprid (**1**) (300 mg/L) in different model solvents (●, methanol; ▲, ethanol; ■, 2-propanol; □, cyclohexane/ethanol (80/20, v/v); ○, cyclohexene/ethanol (80/20, v/v)).

The solutions were purged with nitrogen for 2 min. Irradiation was carried out in water-cooled 40 mL quartz cuvettes (60 mm × 35 mm) with Teflon caps while stirring for up to 7 h using a metal halogen lamp. For HPLC analysis and measurement of UV spectra, samples of 0.5 mL were taken in adequate intervals and evaporated under a stream of nitrogen; the residue was dissolved in 5 mL of methanol.

Following the same procedure, methanolic solutions of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) (300 mg/L) were irradiated.

Solutions of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one oxohydrazone (**6**) in the above-mentioned solvents (300 mg/L) were irradiated in water-cooled quartz glass test tubes (12 mm × 100 mm) for up to 20 min. Samples of 100 μL were taken, diluted with 900 μL of methanol, and analyzed by HPLC.

For the experiments with solid imidacloprid, 500 μL of a methanolic solution (2 g/L) was pipettied onto Petri dishes (diameter 9 cm, without cover). The solvent was evaporated at ambient temperature releasing 1 mg of imidacloprid for each irradiation assay. Irradiations were performed for 24 h in a suntest CPS+ sunlight simulator. For sample preparation, the Petri dishes were rinsed with 10 mL of methanol, and the resulting solutions were subjected to HPLC analysis.

RESULTS AND DISCUSSION

Photodegradation of Imidacloprid. Irradiation of imidacloprid (**1**) in the presence of methanol, ethanol, 2-propanol, cyclohexene, or cyclohexane resulted in nearly the same degradation curves (**Figure 1**). After an irradiation time of 5 h, **1** was completely degraded in all solvents, whereas respective solutions kept in the dark remained unchanged during the same period of time. Previous studies on the photochemistry of pesticides (e.g., parathion (*11*), folpet (*21*), or vinclozolin (*12*)) strongly showed different degradation rates and product distributions depending on the solvent system used. Therefore, it was rather surprising that the photodegradation rate of **1** was not influenced by the solvent environment.

As the main photoproduct, 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) could be isolated by preparative HPLC and was identified by LC/MS and ^{1}H- and ^{13}C NMR spectroscopy. It was formed up to 96 mol% of the degraded imidacloprid (**Table 1**). Additionally, 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one (**2**), which is already known as a hydrolysis product especially under alkaline conditions (*22*), was quantified as a minor photoproduct (**Table 1**).

Contrarily to the photochemical behavior of imidacloprid in water (*3*−*7*), the photolysis in organic solvents is obviously less complex, yielding **5** as main product that is not further photodegraded. However, irradiations in both water (*3*−*7*) and organic solvents only showed reactions at the nitroguanidine moiety but no change of the chloropyridine ring, where dechlorination could be expected (*23*).

Table 1. Formation of the Photoproducts 1-[(6-Chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) and 1-[(6-Chloropyridin-3-yl)methyl]imidazolidin-2-one (**2**) during UV Irradiation of Imidacloprid (**A**, 7 h) and *N*-Nitroso-imidacloprid (**B**, 30 min) in Different Model Solvents

	A		B	
solvent	mol% **5**[a]	mol% **2**[a]	mol% **5**[a]	mol% **2**[a]
methanol	87.9 ± 1.2	3.6 ± 0.2	60.8 ± 5.2	6.2 ± 1.1
ethanol	96.2 ± 1.7	5.8 ± 0.4	76.6 ± 0.7	6.4 ± 0.4
2-propanol	91.2 ± 8.2	6.6 ± 0.8	100.7 ± 8.2	<2 mol%[b]
cyclohexene/ethanol (80/20, v/v)	95.4 ± 6.4	6.1 ± 0.2	86.4 ± 5.4	2.5 ± 1.1
cyclohexane/ethanol (80/20, v/v)	66.7 ± 4.3	6.5 ± 1.4	94.0 ± 5.1	2.4 ± 0.5

[a] $n \geq 3$. [b] Below limit of quantification.

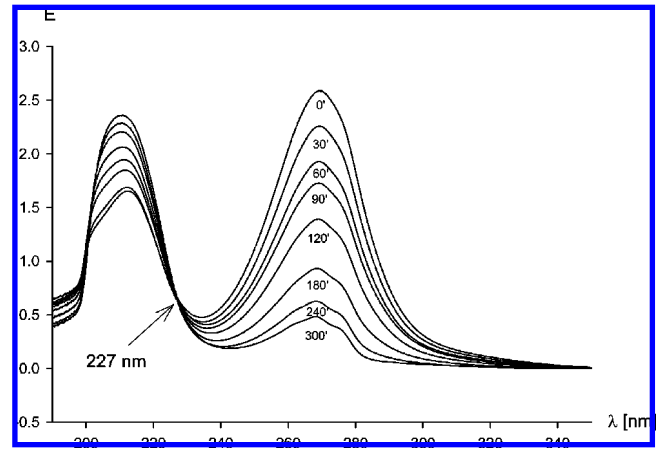


Figure 2. UV spectra of imidacloprid solutions during irradiation (300 mg/L, methanol) for up to 300 min (isosbestic point at 227 nm).

In addition to HPLC measurements, UV spectra of irradiation assays in methanol were recorded. The curves crossed in one point at 227 nm (**Figure 2**). This isosbestic point could be seen as a hint that the reaction is either first order or that an intermediate product possesses a very short lifetime or a low absorbance in the wavelength range recorded (*24*). Thus, the formation of an intermediate with certain photostability seemed unlikely.

A possible reaction mechanism was delivered by Chow et al. (*25*) who carried out photolysis studies with *N*-nitrodialkylnitramines in different solvents such as methanol, *n*-hexane, and acetonitrile in the presence and absence of cyclohexene. These irradiation experiments resulted in the corresponding amino, formamido, or nitroso compounds in different ratios depending on the solvent and the reaction conditions. As in the present study, photoaddition products of *N*-nitrodialkylnitramines and cyclohexene were not obtained. The photolysis is explained by Chow et al. (*25*) by a radical mechanism where in an initial step homolytic cleavage of the N−N bond generates nitrogen dioxide and the aminyl radical, which could undergo further reactions. N−N bond cleavage can also be assumed during the photolysis of **1** resulting in **5** by hydrogen transfer from the organic solvents (**Scheme 2**). Such a mechanism was also proposed by Moza et al. (*4*) for photolysis of **1** in water but with a subsequent hydrolysis of **5** leading to the formation of **2**.

To elucidate the reaction pathway of the formation of **2** and to test if **2** is a photoproduct of **5**, we irradiated solutions of **5**

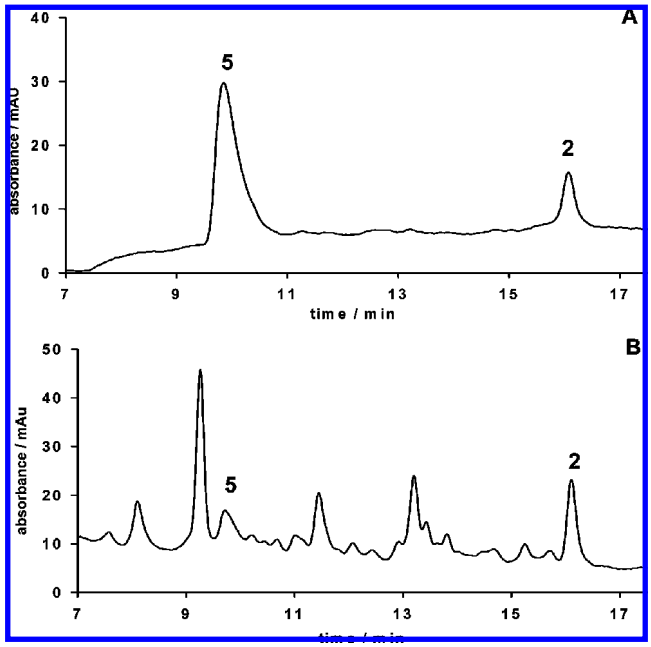


Figure 3. Extended section of HPLC chromatograms (270 nm) of a solution of imidacloprid (**1**) in cyclohexane/ethanol (300 mg/L) after 7 h of irradiation (**A**) and an irradiation assay of solid **1** after 24 h (**B**). **5**: 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine; **2**: (1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-one.

in methanol. After 5 h of irradiation, **5** was not degraded at all, and **2** could not be detected. Consequently, **5** is to be termed as photostable under the chosen irradiation conditions.

A possible intermediate in the formation of **2** is *N*-nitrosoimidacloprid (**6**). Nitrosamines are described as main photoproducts of nitramines (*25*), and during irradiation of **1** on leaves of tomato plants, Scholz and Reinhard (*10*) observed **6** as one of four main photoproducts. But, during the irradiations of **1** in our solvent systems, **6** could not be identified as photoproduct. Therefore, **6** was synthesized, and irradiation experiments were performed to determine the photoreactivity of this possible photodegradation intermediate. Irradiations were carried out in methanol, ethanol, 2-propanol, cyclohexane, and cyclohexene under the same conditions used for **1**. Photodegradation of **6** took place very fast with a half-life of about 3 min in all solvents. Control samples stored in the dark for the same period of time showed no degradation at all. Hence, **6** is rather short-living under the chosen irradiation conditions. As in the case of imidacloprid, **5** was formed as main product in amounts between 61 and 100 mol% (**Table 1**), and **2** was found as minor product. Loss of nitrogen can directly lead to **2** and serve as an explanation for its formation pathway (**Scheme 2**). Other photoproducts as the corresponding formamide as reported for irradiated *N*-nitrosopiperidine (*26*) were not detected. Therefore, during irradiation of **1**, possibly formed **6** will immediately degrade resulting in **2** and **5** and cannot be detected as intermediate.

After spray application of imidacloprid in the field and evaporation of the solvents, a solid residue will first remain on fruit and leaf surfaces. Therefore, further irradiation experiments were carried out with solid **1** on glass as a comparatively inert surface to compare the behavior of solid and dissolved imidacloprid. After 24 h of irradiation, **1** was completely degraded. Contrary to irradiation in solution, the formation of **2** and **5** occurred in rather low amounts (3.3 and 8.1 mol%, respectively), but a variety of additional photoproducts was detected by HPLC (**Figure 3**). All unknown photoproducts having similar UV

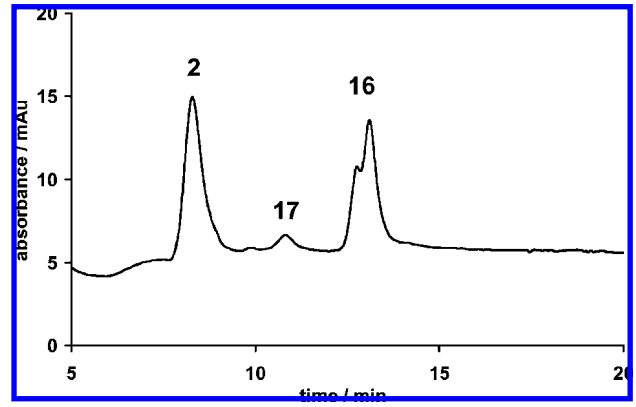


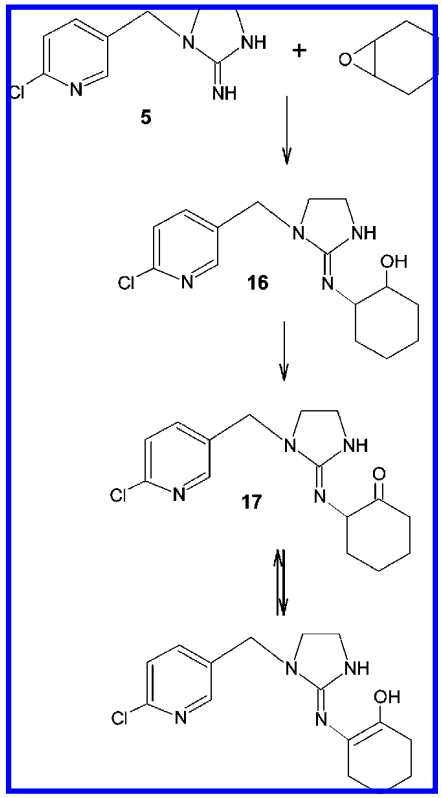
Figure 4. Extended section of an HPLC chromatogram (270 nm) of the reaction batch of 1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-imine (**2**, 5 mg/mL) and cyclohexene oxide (0.25 mg/mL) in methanol, yielding the addition products **16** and **17**.

spectra as **5** were quantified at 270 nm and yielded 36 mol% calculated and expressed as **5**.

The irradiation assays were also analyzed by LC/MS, and some compounds could be tentatively attributed to known photoproducts. Both peaks eluting at 12.1 and 13.6 min delivered protonated molecules of *m*/*z* 226 corresponding to the reported oxidation products **13**, **14**, or **15** (**Scheme 1**) found after irradiation of **1** in water (*3, 4, 7*). An $[M + H]^+$ at *m*/*z* 243 under the peak at 7.5 min can be assigned to **12**, another reported photooxidation product (*7*). Cleavage of the imidazolidine ring of **1** leads to the photoproducts **9** or **11**, both with $[M + H]^+$ at *m*/*z* 171, appearing in the LC/MS chromatogram at 12.4 and 12.9 min. The protonated molecule of *m*/*z* 184, detected at the retention time of 8.1 min, fits with compound **8** or **10**, both reported as photoproducts of **1** (*4, 7*). Only for the protonated molecule at *m*/*z* 242, no photoproduct described in literature could be assigned. A possible photoproduct yielding an $[M + H]^+$ at *m*/*z* 242 is the hydroxylamino derivative of **1** formed by photoreduction of the nitro group. Summarizing, irradiation of solid **1** delivered a totally different and more complex product spectrum than irradiation in organic solvents.

Reaction of Photoproduct 5 with Epoxides. It is known that amines react with epoxides by opening the oxirane ring resulting in β-amino alcohols (*27*). After spiking tomato cuticles with aminoparathion as a well-known photoproduct of parathion and incubation in the dark, it could be shown that aminoparathion was covalently bound to the fruit cuticle (*28*). The formation of nonextractable residues of aminoparathion was explained by reactions with epoxides occurring in cutin acids (*14*). In model studies with cyclohexene oxide and 9,10-epoxystearic acid, the respective reactivity of aminoparathion could be proved (*28*). Since during the present studies the imine **5** was found to be an important photoproduct of **1**, the reactivity of **5** toward epoxides was checked. During incubation of **5** and cyclohexene oxide in methanol at ambient temperature in the dark, 35% of **5** had disappeared after 14 days, and two products could be detected by HPLC whereas the peak of compound **16** was slightly split up (**Figure 4**). LC/MS experiments revealed the expected protonated molecule at *m*/*z* 309 for the main product **16** throughout the double peak, whereas the minor product **17** offered an $[M + H]^+$ at *m*/*z* 307. Both products showed the typical isotope pattern of singly chlorinated compounds. As aminolysis of cyclohexene oxide should only deliver the corresponding *anti*-amino alcohol (*29*), splitting up of the main peak can only be attributed to the formation of E/Z isomers

Scheme 3. Reaction of 1-[(6-Chloropyridin-3-yl)methyl]imidazolidin-2-imine (**5**) with Cyclohexene Oxide



at the carbon nitrogen double bond. Although this should also hold true for the minor product **17**, it could not be observed by HPLC.

From a preparative assay, **16** was isolated by preparative HPLC in an amount of about 50%. The determined exact mass $[M + H]^+$ of 309.1472 confirmed the molecular formula of $C_{15}H_{21}N_4OCl$ corresponding to an [1 + 1] addition product of **5** with cyclohexene oxide (**Scheme 3**). The NMR spectra of the mixture clearly showed both signals of aromatic protons at 8.39, 7.84, and 7.54 ppm belonging to the pyridine part of the molecule and signals of methylene protons in the area between 1.24 and 2.16 ppm and 3.21 and 3.55 ppm, which can be attributed to the protons of the cyclohexane ring. The exact mass of the minor product **17** of 307.1321 ($[M + H]^+$) and the quite similar NMR spectra as obtained for **16** support **17** as an oxidation product of **16** (**Scheme 3**).

The same reactivity of **5** was found in the presence of 9,10-epoxystearic acid occurring as 18-hydroxy-9,10-epoxystearic acid in several plant cuticles (*14*). LC/MS screening of incubation experiments with **5** and methyl 9,10-epoxystearate afforded products with $[M + H]^+$ at *m/z* 523 and 521 proving the formation of an addition product that partly underwent oxidation during incubation.

Concludingly, imidacloprid is rather susceptible to photodegradation independent upon the chemical environment, resulting in **5** as main product in organic solvents but without the formation of photoaddition products with solvent molecules. However, photoproduct **5** is quite reactive toward epoxidized cutin acids supporting a possible pathway for the formation of nonextractable residues of imidacloprid in plants, which were described in the literature. Therefore, further research is needed to transfer the results from model systems to plant surfaces.

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