

Environmental Fate of Trifloxystrobin in Soils of Different Geographical Origins and Photolytic Degradation in Water

KAUSHIK BANERJEE,[†] AXEL PATRICK LIGON,[‡] AND MICHAEL SPITELLER^{*‡}

National Research Centre for Grapes, Pune 412 307, India, and Institute of Environmental Research (INFU), University of Dortmund, Otto-Hahn-Strasse 6, 44221 Dortmund, Germany

In vitro biodegradation of trifloxystrobin (TFS) under darkness could best be explained by two-compartment first + first-order rate kinetics with half-lives ranging between 1.8 and 2.3 days. Hydrolysis was found to be the major pathway of degradation resulting in the formation of the acid metabolite, TFS-acid, with an EE conformation. The adsorption rate kinetics of both TFS and TFS-acid followed linear and Freundlich isotherms. The extent of adsorption was directly correlated with organic matter and clay contents, whereas desorption had a negative correlation. The high partition coefficients (K_D) indicate strong adsorption of TFS on all of the test soils without any appreciable risk of groundwater contamination. In case of the TFS-acid, however, the adsorption was weaker; hence, if its further degradation is slow, it may contaminate lower soil horizons under worst case conditions. TFS did not cause any adverse effect on the soil microbial population. TFS was susceptible to aquatic photolysis in summer with an environmental half-life of 0.7–1.3 days irrespective of the latitudes.

KEYWORDS: Trifloxystrobin; soils; biodegradation; half-life; adsorption; desorption

INTRODUCTION

Trifloxystrobin {methyl- α -(methoxyimino)-2-[(1-[3-(trifluoromethyl)phenyl]ethylidene)amino]oxymethyl]benzeneacetate} (TFS) is a systemic fungicide marketed by Bayer Crop Science (1). It is the first strobilurin compound with an oximether side chain. The combination of the methyl and substituted phenyl substituents in the oximether moiety and the presence of the trifluoromethyl group in the side chain phenyl moiety reportedly provides a broad fungicidal bioefficacy against a variety of crop pests (2, 3).

A close look at the chemical structure of TFS (=EE isomer) indicates that this molecule can appear in the form of at least four geometrical isomers, namely, EE (1), EZ (2), ZE (3), and ZZ (4) (**Figure 1**). We have reported the X-ray crystallographic structures of the ZZ, ZE, and EZ isomers earlier (4–6). The ZZ, ZE, and EZ isomers have significantly less biological activity as compared to the EE isomer (7). Furthermore, because TFS is an ester, it is susceptible to hydrolysis, which results in the formation of the corresponding acid metabolite with no bioefficacy against target pests like *Botrytis cinerea* (8, 9). The X-ray crystallographic structure of the TFS-acid produced by the alkaline hydrolysis of TFS has been reported by us (10).

The present project was undertaken to study the fate of TFS in soil and aquatic environments. Soil is known to be a sink for every chemical applied in agriculture. During recent years,

increasing concern has been voiced about the pollution of soil and aquatic environments with pesticides. A reason for this concern stems from the evidence that pesticide residues in the environment can traverse into a dynamic system to another, degrade in situ, or move out of a system to another, endangering the health or well being of human beings and natural resources. Residues in soils after harvesting of a crop often persist for a long period and contaminate the next crop, groundwater, and food chain as a whole. TFS has very low water solubility (610 $\mu\text{g/L}$ at 25 °C), but its primary metabolite, being an acid, is soluble in water and hence may emerge as a potential contaminant of groundwater. When TFS is applied in agriculture, on exposure to the open environment, the biologically active isomer EE is susceptible to be converted to a mixture of four isomers, which further may get hydrolyzed to the corresponding acid metabolites. All of these conversion products are biologically inactive and may lead to excessive application of this fungicide to achieve the desired level of pest control. Thus, to develop an effective strategy for the management of TFS residues in environment, it is a prerequisite to explore its postapplication fate with regard to the isomerization behavior and rate of degradation in soil. As the above processes essentially depend upon the physicochemical and microbiological properties of soil, it is, therefore, important to study the fate of TFS in soils of different geographic origins and physicochemical properties.

Furthermore, knowledge on the sorption behavior of TFS in soils is also necessary to assess the risk of groundwater contamination as a consequence of controlled or uncontrolled distribution of TFS in the soil environment. The risk of groundwater contamination largely depends on the partitioning

* To whom correspondence should be addressed. Tel: +49 231 755 4080. Fax: +49 231 755 4085. E-mail: m.spiteller@infu.uni-dortmund.de.

[†] National Research Centre for Grapes.

[‡] University of Dortmund.

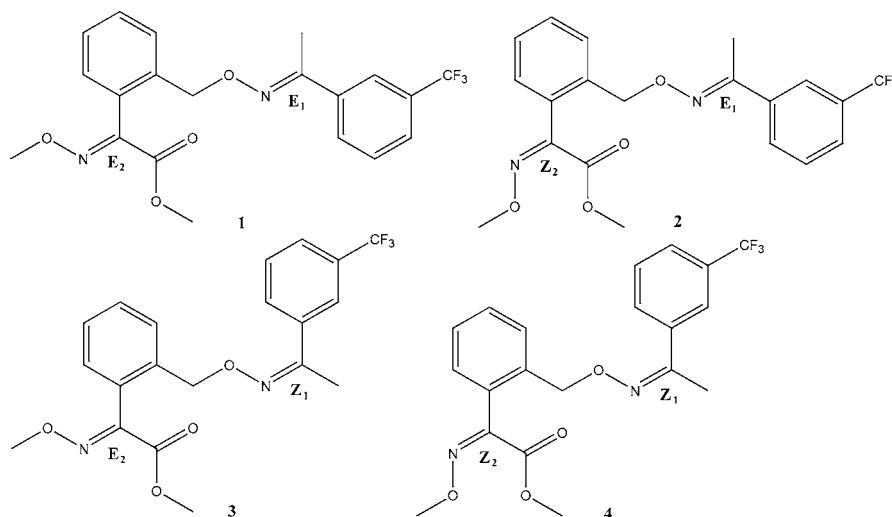


Figure 1. Four geometric isomers of TFS.

Table 1. Physicochemical Characteristics of the Test Soils

soil	Ashoknagar	Pune	Monheim
texture class	silty clay	clay	sandy loam
sand (2000–50 μm) (%)	11	18	72
silt (<50–2 μm) (%)	47	22	23
clay (<2 μm) (%)	43	53	5
pH (water, ratio 1:2.5)	7.0	8.3	6.75
organic carbon (%)	3.8	5.5	1.69
cation exchange capacity (mequiv/100 g)	26.2	47.5	8.0
maximum water holding capacity (%)	42.5	85	34.4
density (g/mL)	1.5	1.9	2.5
soil class (U.S. Department of Agriculture)	Typic Ustochrepts	Vertic Ustropepts	Typic Cambudolls
origin	Ashoknagar, District-North 24 Parganas, West Bengal, India	NRC Grapes experimental farm, Pune, Maharashtra, India	Bayer Crop Science experimental farm, Monheim, Germany

of an applied pesticide between the solid and the liquid phases of the soil system. Hence, it was decided to study the adsorption–desorption of TFS and TFS-acid in all of the soils, where degradation experiments were conducted (11).

This research paper presents the results of the investigations on the isomerization, degradation, and sorption behavior of TFS in soils of different geographic origins and different physicochemical properties to understand its behavior in the soil environment. The persistence of TFS in aquatic environments in different seasons and locations was also estimated to assess the risk of environmental contamination.

EXPERIMENTAL PROCEDURES

Chemicals. The analytical standard grade TFS and the internal standard, kresoxim-methyl [methyl (αE)- α -8-methoxyimino]-2-[(2-methylphenoxy)methyl]benzeneacetate] were of >99% purity and were purchased from Ehrenstorfer GmbH (Germany). All of the solvents used in the experiment were of high-performance liquid chromatography (HPLC) grade.

Soil. Two soils originating from India and one soil originating from Germany having different physicochemical properties were included in the present study. Each soil sample was collected from the top soil layer of 0–10 cm. From India, the soil samples were shipped immediately after collection by airfreight to Germany, maintaining their original moisture status. Prior to the laboratory experiments, the soils were air-dried and sieved to a maximum particle size of <2 mm. The physicochemical properties are shown in Table 1. None of the soils investigated had been treated with TFS in the previous 5 years.

Table 2. Activation Parameters of the Alkaline Hydrolysis of TFS

solvent	acetonitrile/ NaOH (0.1 M) 1:1 (v/v)	acetone/ NaOH (0.1 M) 1:1 (v/v)	methanol/ NaOH (0.1 M) 1:1 (v/v)
E_a (kJ mol ⁻¹)	99.9	69.2	11.2
$\ln A$	37.9	27.0	3.3
ΔH^\ddagger (kJ mol ⁻¹)	97.4	66.7	8.7
ΔS^\ddagger (J mol ⁻¹ K ⁻¹)	62	-29	-226
$\Delta G_{303.15}^\ddagger$ (kJ mol ⁻¹)	78.6	75.5	77.2

Activation Parameters of the Alkaline Hydrolysis. The activation parameters pertaining to the alkaline hydrolysis of TFS were determined. Two milligrams of TFS was dissolved separately in 1 mL of acetonitrile, acetone, and methanol and mixed thoroughly with 1 mL of 0.1 M NaOH. All of the solutions were preheated at 20, 30, or 40 °C. The progress of hydrolysis was continuously monitored by HPLC. For each sample, the neutral pH was maintained by the addition of 0.01 M HCl before injection into HPLC. On the basis of the Arrhenius and Eyring equations, the activation parameters of the alkaline hydrolysis of TFS were calculated (Table 2).

Application of TFS in Soil. The experimental setup was designed on the basis of the EC Directive (12), the SETAC procedures (13), the BBA guideline (14), and the FDA Technical Assistance Handbook (15). TFS was mixed into the soils at the recommended commercial use rate of 500 g active ingredient/ha. To avoid any effects of the solvents upon the biological activity of the soils, the calculated volumes of the application solution in methanol were initially dispensed onto portions of about 60 g of air-dry soil in porcelain dishes. The treated subsamples

of soils were thoroughly mixed with a spatula until the solvent was completely evaporated (ca. 10 min) and afterward added to the total mass of the corresponding soils. Subsequently, the gross mass of each soil was mixed in a tumbling mixer for 1 h. This was followed by aliquotation. Batches of 100 g of soil each (relative to dry soil) were incubated in Erlenmeyer flasks under controlled temperature (20 ± 1 °C) in darkness. The moisture content of each soil was adjusted to 60% of its maximum water holding capacity and gravimetrically checked once every 2 weeks.

Soil Biological Property. The microbiological activity in soils was determined with and without the addition of chemicals at the beginning and the end of the experiment on the basis of the respiration curve of the soil microflora (16). The application of the active ingredient and the subsequent incubation of the soil samples used for the determination of microbial biomass (C_{mic}) were performed by using the procedure previously described for the soil incubation procedure.

Sampling. The samples were drawn 0, 1, 3, 7, 14, 31, and 90 days after application for analysis.

Recovery, Extraction, and Quantitative Determination of the Residues. The recovery experiments were carried out on untreated soils by fortifying with TFS, its isomers, and TFS-acid at 10 ng/mL level. EZ-acid, ZE-acid, and ZZ-acid were simultaneously spiked in the same sample at 100 ng/mL level. The samples were exhaustively extracted five times at room temperature on a mechanical shaker, once with 108 mL of acetonitrile–water (100/8) and four times with 100 mL of acetonitrile–water (80/20). Each time, the samples were shaken for 45 min. After each extraction step, the extracts were separated from soil solids by decantation and filtration and pooled. The combined extract was evaporated to complete dryness on a water bath at <40 °C. The residue was dissolved in 1 mL of acetonitrile for chromatographic measurement.

Quantitative determination of TFS, its isomers, and the four acid metabolites in soil was done by liquid chromatography–tandem mass spectrometry (LC-MS/MS) selective reaction monitoring (SRM) method. All of the analytes were chromatographically separated with baseline separation. Because deuterated or ^{14}C -labeled TFS was not available, kresoxim-methyl, another strobilurin fungicide having comparable structure to TFS, was used as the internal standard.

The operating parameters were as follows: Dionex HPLC, column Phenomenex RP-18, 3 μm , 150 mm \times 2 mm i.d.; eluent A, Milli-Q $\text{H}_2\text{O}/\text{HCOOH}$ (1 mL/L), and eluent B, $\text{CH}_3\text{CN}/\text{HCOOH}$ (1 mL/L); flow rate, 0.2 mL/min; gradient, 0 min/40% B to 10 min/70% B to 19 min/88% B to 20 min/100% B to 33 min/40% B; UV diode array detector set at a maximum absorbance of 250 nm.

HPLC-MS. TSQ 7000 (Finnigan MAT, Bremen, Germany) equipped with an APCI source. For APCI, a vaporizer temperature of 450 °C and a transfer capillary temperature of 230 °C were used. The ionization current and detector voltage were set to 5 μA and 1.3 KV, respectively. For SRM scans, a dwell time of 400 ms and a scanning time of 0.5 s were applied.

Degradation Kinetics. Many published degradation studies assumed simple first-order degradation kinetics. Often, the degradation of pesticides in soil does not follow simple first-order kinetics but shows a pattern where the pesticide declines at an initial phase rapidly and in a second phase less rapidly. To describe the nonlinear decline of the pesticides residue in soil, a number of nonlinear equations, like the square root first-order model or the first + first-order model, were developed (17–19).

The following mathematical expressions are used in this study. First-order model:

$$[A]_t = [A]_1 \cdot \exp(-k_1 \cdot t) \quad (1)$$

Square root first-order model:

$$[A]_t = [A]_1/k_1 \sqrt{t} \quad (2)$$

First + first-order model:

$$[A]_t = [A]_1 \cdot \exp(-k_1 \cdot t) + [A]_2 \cdot \exp(-k_2 \cdot t) \quad (3)$$

where $[A]_t$ is the concentration ($\mu\text{g kg}^{-1}$ soil) of A at time t (days) and $[A]_{1,2}$ are the initial concentrations of A at time 0 degraded through a first or square root first-order process and another first-order process. $k_{1,2}$ are the degradation rate constants 1 and 2. The units of k depend on the model used. The rate constant k is given in the unit days^{-1} in the first order as well as in the first + first-order model. In the square root first-order model, however, numerical values of k (without a unit) are used as a consequence of the equation being only empirical (17). The time at which the concentration reaches the half of the initial concentration is the half-life $t_{1/2}$ or DT_{50} (time of 50% degradation). The substitution into the above models gives the following. First-order model:

$$t_{1/2} = \ln 2/k_1 \quad (4)$$

Square root first-order model:

$$t_{1/2} = (\ln 2/\ln k_1)^2 \quad (5)$$

The first + first-order model cannot be described in a differential form; for this reason, DT_{50} can only be calculated by an iterative procedure. The equation parameter ($[A]_{1,2}$, $k_{1,2}$, R^2 , and DT_{50}) would be calculated by use of a commercially available program, namely, OriginPro (20), which allows the definition of new equations and statistical parameters.

Preparation of the Isomers of TFS and the Corresponding Acid Metabolites. The isomers of TFS, namely, EZ, ZE, and ZZ, were prepared by irradiating the pure EE isomer in acetone at 500 W/m^2 in a quartz vessel placed inside a Suntest (Heraeus) apparatus. The individual isomers were purified by preparative HPLC. Each of the isomers was obtained in pure crystalline form (4–6). The monoacid metabolites of EE, EZ, ZE, and ZZ conformations were separately prepared by alkaline hydrolysis of the individual esters with 0.05 M NaOH at room temperature under continuous stirring. The progress of the hydrolysis was continuously monitored by HPLC. After complete hydrolysis, the pH of the reaction solution was neutralized by 0.01 M HCl and then evaporated at <40 °C until the white solids separated out. The white solids were identified by comparison with the reference monoacid standards.

Method Validation. The soil samples were analyzed for the residues of the four isomers, namely, EE, EZ, ZE, and ZZ, and their corresponding four monoacid metabolites, i.e., TFS-acid (EE), EZ-acid, ZE-acid, and ZZ-acid. In all of the soils, the recoveries of TFS, TFS-acid, and three isomers of TFS were $>99\%$. The recoveries of the other three compounds were $>80\%$. The residues of each compound in the soil extracts were simultaneously identified by LC-MS/MS and also on the basis of their retention times (RTs). The quantification was based on the calibration obtained by plotting the area ratio of the daughter ion of the individual target compound to that of the internal standard vs concentration of the calibration standard. The limit of detection (LOD) was based on a signal-to-noise ratio greater than 3.

Theoretical Photolysis Half-Life of TFS. The theoretical environmental half-life pertaining to the photolysis of TFS in aquatic environment was calculated as a function of the half-life in the Suntest unit $\text{DT}_{50}^{\text{Suntest}}$, the Suntest radiation S_R , and the global radiation G_R at different locations (21).

$$\text{DT}_{50}^{\text{Enviro}} (\text{day}) = \text{DT}_{50}^{\text{Suntest}} (\text{h}) \cdot \frac{S_R (\text{MJ/m}^2 \text{ h})}{G_R (\text{MJ/m}^2 \text{ day})}$$

The light intensity (in W/m^2) of the artificial light source in the Suntest unit was measured by a radiometer at 290–800 nm. Around 57.9% of the irradiance of the global radiation (290–3000 nm) falls in the range of 290–800 nm (21). Thus, the Suntest light intensity has to be multiplied by 100%/57.86% and with 3600 s to get the radiation of a Suntest unit during 1 h.

The Suntest half-life pertaining to the three different liquid media, namely, pure acetone, water/methanol (9/1 v/v), and water/methanol (9/1 v/v) + humic acid 10 mg/L was used. The aqueous solutions did not contain any photosensitizer like acetone or acetonitrile. Half-lives

at 12 different geographic locations situated between the latitudes of 20° and 60° N in the months of March, June, September, and December representing spring, summer, fall, and winter seasons, respectively, were estimated.

Sorption Study. The adsorption–desorption study was conducted using U.S. EPA, designation CG-1710 (22). Because all pesticides are used in agriculture as formulations and the adsorption–desorption behavior of a pure pesticide may not necessarily represent the behavior of its formulated preparations (11), it was decided to study the adsorption–desorption experiments with the water dispersible agricultural formulation of TFS, namely, Flint 50WG containing 50% active ingredient. A 0.01 M solution of calcium chloride was used as an aqueous phase, to make phase separation easier and to simulate an ionic strength similar to that of a natural soil solution. All test substances were dissolved in this solution to obtain the desired dilutions. To minimize biological activity, 0.0001 M HgCl₂ was added. The stock solution containing 1000 mg/L of TFS was prepared by dissolving 2000 mg of flint (50% TFS) in 1 L of CaCl₂ solution with thorough stirring. The stock solution of the acid metabolite (1000 mg/L) was prepared by dissolving pure TFS-monoacid in CaCl₂ solution. The solutions were sonicated for 10 min for complete dissolution. The application solutions of TFS and TFS-acid were prepared by diluting the stock solutions with CaCl₂ to the levels of 10.0 and 6.8 mg/L as measured concentrations by LC-MS/MS. Soil (10 g), and 20 mL of TFS solution and 10 mL of TFS-acid solution (solid/liquid ratio 1:2 and 1:1, respectively) were placed in 50 mL stoppered glass centrifuge tubes and shaken on an overhead shaker for 24, 48, and 72 h at 22 ± 1 °C. At the end of each shaking period, the tubes were removed and centrifuged at 2800 rpm for 20 min. The clear supernatants were decanted, measured for volume, and analyzed directly by LC-MS/MS for the concentration of TFS and TFS-acid. The amount of test substance adsorbed was computed by the difference between the initial and the equilibrium concentrations in the aqueous phase.

The adsorption isotherms were determined using five initial concentrations ranging from 0.14 to 10.15 mg/L of TFS and TFS-acid in same solution with a shaking period of 48 h. All treatments were performed in duplicate. Blank samples containing only the solutions of the test substances without any soil were run in parallel.

The desorption study was carried out by adding an extra amount of freshly prepared 0.01 M CaCl₂ solution to the soil remaining in the centrifuge tubes while maintaining the total amount of the solution to 20 mL gravimetrically. The centrifuge tubes were again shaken on an overhead shaker for 48 h, decanted, and analyzed by HPLC for the concentrations of TFS-acid and TFS separated at the RTs of 14.7 and 18.2 min, respectively.

Calculations. The adsorption–desorption data were fitted into the following linear forms of isotherms (23).

$$q = K_D C_e \quad (6)$$

$$\log q = \frac{1}{n} \log C_e + \log K_F \quad (7)$$

$$\frac{1}{q} = \frac{1}{K_1 K_2} \cdot \frac{1}{C_e} + \frac{1}{K_1} \quad (8)$$

Here, $1/K_1 K_2$ is the slope and $1/K_1$ is the intercept of the resulting straight line; q = a variable that represents the concentration of the adsorbed compound ($\mu\text{g/g}$ soil); C_e = equilibrium concentration of the test substance ($\mu\text{g/mL}$); K_D = linear or distribution adsorption constant (mL/g); and $1/n$ and K_1 are the constants reflecting the adsorption strength or intensity.

K_F and K_2 represent the adsorption capacity for the Freundlich and Langmuir equations, respectively. K_D is related to the soil organic carbon (OC) and soil organic matter (OM) by the following equations (24):

$$K_{OC} = 100 \cdot K_D / \%OC \quad (9)$$

$$K_{OM} = 100 \cdot K_D / \%OM \quad (10)$$

Table 3. SRM for TFS Isomers and Acid Metabolites

name	retention time (min)	M ⁺ (m/z)	daughter ion (m/z)	collision energy (eV)	LOD (ng/mL)
EE (TFS)	18.2	409	185.8	−22	<10
EE-acid (TFS-acid)	14.7	321	117.8	−23	10
EZ	16.9	409	205.8	−20	<10
EZ-acid	13.6	395	147.7	−22	30
ZE	20.9	409	185.8	−22	<10
ZE-acid	16.5	395	185.7	−22	50
ZZ	19.3	409	205.9	−23	<10
ZZ-acid	15.0	395	147.8	−20	30
kresoxim-methyl (internal standard)	15.7	313.9	221.9	−22	

Because %OM/%OC = 1.724 (19), K_{OC} could be calculated by the following equation:

$$K_{OC} = 1.724 K_{OM} \quad (11)$$

RESULTS AND DISCUSSION

On closer examination of the activation parameters (Table 2), a decrease in the values of E_a , $\ln A$, ΔH^\ddagger , and ΔS^\ddagger is evident in the order acetonitrile > acetone > methanol. $\ln A$ and ΔS^\ddagger measure the order in each system. Moving from acetonitrile to methanol, the polar transition state complex formed between TFS and OH[−] is increasingly solvated. Between the initial state and the transition state, there is no change in translational and rotational degrees of freedom. The parameters E_a and ΔH^\ddagger measure the amount of binding energy lost in the formation of the complex. The increase in solvation in the transition state leads to a decrease in ΔH^\ddagger .

All four isomers of TFS and the corresponding four acid metabolites could be chromatographically separated with baseline separation. The RT, SRM data, and LOD of all of the compounds are presented in Table 3.

The average environmental half-life of TFS in the reaction medium comprising water/methanol (9/1 v/v) + humic acid 10 mg/L in June at all locations (20–60° N) was 1.1 days. In all photolysis studies, the main pathway was isomerization. The theoretical environmental half-lives, calculated at 12 different locations in four different seasons in water, are presented in Table 4. The data indicate that even in summer, the TFS molecule takes 0.7–1.3 days to degrade by 50% irrespective of the latitudes. The half-life extends with the seasonal change from spring to winter. This finding indicates that TFS is susceptible to photolysis.

Persistence of TFS in Soils. The extractable residues of TFS and TFS-acid in the soils are presented in Table 5. The kinetics of the residue data of TFS was evaluated by fitting the data into first, square root first, and first + first-order kinetic models, which were further compared for the best fit (Table 6). In all of the soils, the dissipation rate was faster at the beginning, which slowed down with passage of time. The nonlinear pattern of degradation indicates that simple first-order kinetics is not adequate to explain the dissipation of TFS in soil. In all of the soils, the fit of the data to the first-order kinetics was relatively poor as compared to the other two models with $R^2 < 0.90$ for Pune and Monheim soil and <0.97 for Ashoknager soil with a too great half-life in each case. The typical nature of dissipation suggests the fit of data to a two-compartment first + first-order kinetics model, where one part of the added TFS, which is immediately available in soil solution phase, degraded rapidly, leaving the other part possibly remaining adsorbed on the adsorbing sites comprising organic and clay fractions (19). The

Table 4. Estimated Environmental Half-Life (DT₅₀) of TFS

location	latitude	environmental DT ₅₀ (days) ^a			
		March	June	September	December
San Juan, Puerto Rico (United States) ^b	18°26' N	1.1	1.0	1.1	1.5
Calcutta, India ^b	22°32' N	1.0	1.2	1.3	1.4
Mobile, AL (United States) ^b	30°41' N	1.3	1.0	1.3	2.3
Phoenix, AZ (United States) ^b	33°26' N	1.0	0.7	0.9	1.9
Tokyo, Japan ^b	35°11' N	1.8	1.5	2.1	2.6
Athens, Greece (European Union) ^b	38°03' N	1.9	1.1	1.5	5.3
Zürich, Switzerland ^b	47°23' N	2.3	1.2	1.8	7.0
Vancouver, BC (Canada) ^b	49°15' N	2.1	1.0	1.6	10.6
London, Great Britain (European Union) ^b	51°30' N	3.0	1.3	2.1	10.6
Berlin, Germany (European Union) ^c	52°31' N	2.2	1.0	1.7	14.0
Copenhagen, Denmark (European Union) ^d	55°41' N	2.2	0.9	1.7	14.1
Stockholm, Sweden (European Union) ^e	59°20' N	1.7	0.9	3.1	21.3

^a DT₅₀^{Suntest} = 6.79 h water/methanol (9/1 v/v) + humic acid 10 mg/L at 500 W/m². ^b For the global radiation, see ref 21. ^c For the global radiation, see ref 40. ^d For the global radiation, see ref 39. ^e For the global radiation, see ref 41.

Table 5. Mean Concentrations of TFS and Its TFS-Acid in Soils with Time

soil/ day	Ashoknagar		Pune		Monheim	
	TFS ($\mu\text{g}/\text{kg}$)	TFS-acid ($\mu\text{g}/\text{kg}$)	TFS ($\mu\text{g}/\text{kg}$)	TFS-acid ($\mu\text{g}/\text{kg}$)	TFS ($\mu\text{g}/\text{kg}$)	TFS-acid ($\mu\text{g}/\text{kg}$)
0	319.9	1.5	247.4	2.4	217.9	2.3
1	196.7	3.2	192.4	19.9	140.4	9.3
3	140.2	10.9	94.3	67.9	94.2	17.4
7	99.0	29.5	49.7	186.5	54.8	22.5
14	56.1	30.8	27.7	235.5	27.7	32.4
31	32.8	6.3	13.7	198.7	9.3	49.8
90	1.90	BDL ^a	3.4	140.5	2.2	44.6

^a BDL, below detectable level.

Table 6. Degradation Parameters for the Analyzed TFS

soil	parameters	unit ^a	first order	Sqrtfirst order	first + first order
Ashoknager	[A] ₁	$\mu\text{g}/\text{kg}$	178.8	317.1	162.57
	k ₁	days ⁻¹	0.05	1.58	1.16
	[A] ₂	$\mu\text{g}/\text{kg}$			156.95
	k ₂	days ⁻¹			0.0632
	R ²		0.96	0.99	0.99
	DT ₅₀	days	13.4	2.3	1.8
Pune	[A] ₁	$\mu\text{g}/\text{kg}$	104.69	260.47	210.80
	k ₁	days ⁻¹	0.043	1.69	0.3870
	[A] ₂	$\mu\text{g}/\text{kg}$			40.74
	k ₂	days ⁻¹			0.0322
	R ²		0.81	0.96	0.99
	DT ₅₀	days	16.2	1.8	2.3
Monheim	[A] ₁	$\mu\text{g}/\text{kg}$	96.81	221.47	97.20
	k ₁	days ⁻¹	0.047	1.66	1.088
	[A] ₂	$\mu\text{g}/\text{kg}$			120.47
	k ₂	days ⁻¹			0.105
	R ²		0.86	0.99	0.99
	DT ₅₀	days	14.9	1.9	2.0

^a In the square root first-order model, k₁ doesn't have a unit (17).

adsorbed fraction subsequently released into the solution phase and dissipated at a relatively slower rate. An excellent fit to the first + first-order model was observed for all of the soils with R² > 0.99 in each case (Figure 2). The dissipation in

Ashoknagar and Monheim soils also could be explained by the empirical square root first-order model, but it gave relatively poor fit for the Pune soil (Table 6). The first + first-order model also gave the most realistic half-life value as compared to the other two models.

The different half-lives in different soils can be attributed to the effect of different physicochemical properties of the test soils on TFS. Furthermore, the rate of degradation might also differ as a consequence of the exposure of TFS to different nature of microbial population in different soils and also to the variability in activity of specific microbial species taking part in the degradation of TFS. Furthermore, on comparing the DT₅₀ values to the theoretical half-life data (Table 4), it is understood that degradation of TFS in soil environment predominantly occurs as a result of its exposure to microbial activity. Although in the present study we did not identify the specific microbial strains responsible for the degradation of TFS, it needs to be investigated in future. The initial faster rate of degradation might be a reflection of the higher microbial biomass at the beginning. At the later phase, after 15 days, the rate of degradation slowed down, which is in agreement with the low microbial biomass observed at the end of the experiment (Table 7).

In all of the samples, the concentrations of ZE, EZ, and ZZ were negligible, which might be because of the nonexposure to light during the experiment. TFS-acid was identified as the major metabolite. Hence, hydrolysis could be the major route of degradation. The concentration of TFS-acid gradually increased with time until 14 days in Ashoknagar and Pune soil, whereas in Monheim soil, TFS-acid residues reached the maximum level on the 31st day (Figure 3). After this, TFS-acid residues decreased, indicating its subsequent degradation to the unidentified metabolites. The maximum build-up of TFS-acid was observed in Pune soil, indicating significant hydrolysis of TFS to its acid metabolite.

Effect on Soil Biological Property. Organic substances of any kind, which are reported to be persistent, are degraded by microorganisms (25). The microbial activity of a test soil is particularly an important factor to study the degradation and metabolism of agrochemicals in soils (26–28).

In the present investigation, the biological property of soils in terms of microbial biomass (mg C_{mic}/kg soil dry weight basis) is listed in Table 7. In all soils, a decline in microbial biomass was observed at the end of the experiment. The same trend was also observed in the control soil without TFS application. This may be attributed to the influence of the incubation period rather than to the effect of TFS itself. During the entire experimental period, the microbial population in soil samples was not supplied with any food and energy from any external source. With the passage of time, a similar phenomenon of decreasing microbial biomass in soil was also demonstrated with different pesticides (29). However, there are many reports regarding the favorable effect of pesticides on the growth and activities of microorganisms in soil (30–33). Furthermore, it has been shown in many studies with the plant protection products that the degradation under field conditions is significantly faster in comparison to laboratory studies. Therefore, the present experiment clearly shows the limitations of soil degradation studies under laboratory conditions, and it may be unwise to extrapolate laboratory results to that of the natural environment.

Adsorption Isotherm. Adsorption of TFS attained equilibrium within 24 h of contact time in Monheim soil, whereas in Pune and Ashoknagar soils, the equilibrium was reached within 24–48 h, as observed from the results of the kinetic studies. In case of TFS-acid, however, the equilibrium was attained after

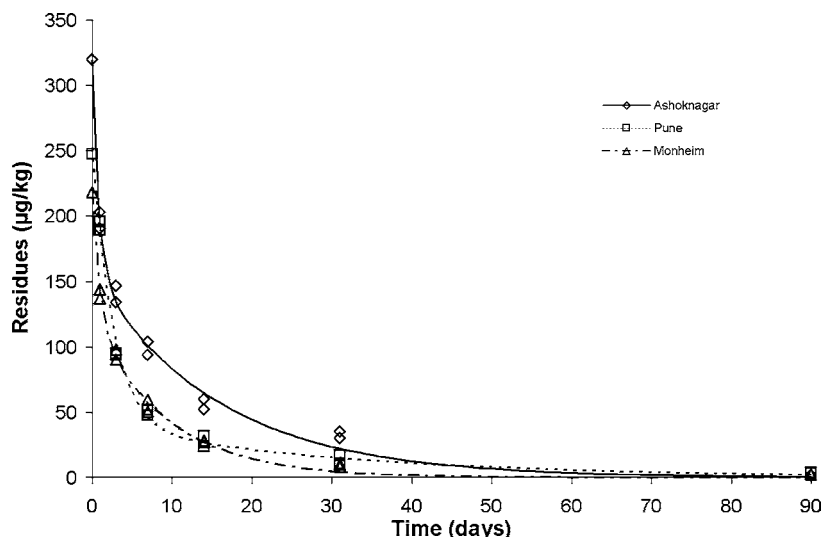


Figure 2. Degradation of TFS in different soils.

Table 7. Effect of TFS on the Microbial Properties of the Test Soils

soil	microbial biomass (mg C_{mic} /kg soil)		
	start	end (treated)	end (untreated)
Ashoknagar	224	183	149
Pune	264	148	166
Monheim	149	106	136

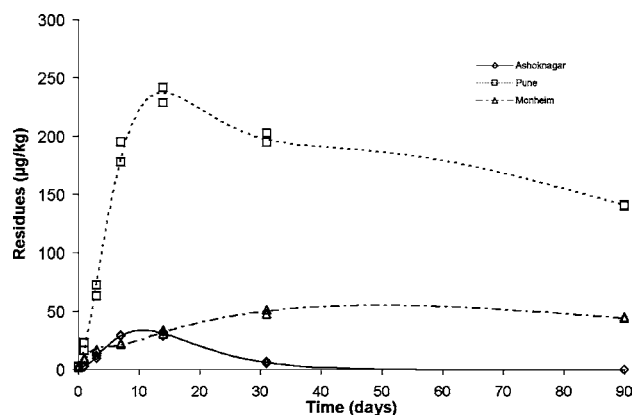


Figure 3. Persistence of TFS acid in three different soils.

24 h. The adsorption equilibrium mentioned here is defined as the time at which the bound concentrations varied less than 5% between two consecutive time measurements (34).

Adsorption occurred in two phases. The first phase, up to 24 h, had a rapid transfer of the test substances from the aqueous phase to the adsorbing sites. This was followed by a slow phase, which is explained by the requirement of more energy for adsorption in less accessible sites. The longer equilibration time in Indian soils could be correlated to the presence of more binding sites in terms of organic matter and clay in both of the soils. The overall amount of adsorption was also, thus, more in Indian soils.

Adsorption of TFS was greater than TFS-acid in the test soils (Table 8). For TFS, the results indicate a good fit to the linear and Freundlich isotherms ($R^2 > 0.97$), whereas for TFS-acid, the best fit was observed to the linear model. In Ashoknagar and Pune soils, the Freundlich model also could explain the adsorption of TFS-acid ($R^2 > 0.99$), but it showed a poor fit for Monheim soil (Table 9).

A good fit to the Langmuir model was observed for the adsorption of TFS in all of the test soils, but as the parameters K_1 and K_2 were negative (Table 9), the Langmuir model could not explain the adsorption of TFS or TFS-acid (35). The nonapplicability of the Langmuir equation to the results might be due to the heterogeneity and surface acidity of these soils. Furthermore, the Langmuir isotherms are generally observed when there is enough of a chemical to form multiple layers on the solid surfaces. In the present studies, the concentration of TFS was quite low and this may be the reason why this model did not work.

The linear adsorption coefficient K_D was higher in Ashoknagar and Pune soils. K_D is the partition coefficient between soil and aqueous phases and is conventionally considered to assess the risk of contamination to groundwater. The higher K_D in the Indian soils may be attributed to the higher organic matter and clay contents (36). This finding is further supported by the higher K_{OC} value observed for the Monheim soil. The K_{OC} values suggest that adsorption of TFS in the test soils is generally strong indicating low leaching potential.

The K_F for Indian soils was more than that of Monheim soil. The parameter K_F in the Freundlich model represents the amount of a chemical adsorbed at an equilibrium concentration of 1 mg/L. K_F in Pune > Ashoknagar > Monheim, and the pattern is the same as for the adsorption data (Table 8). For TFS-acid, a lower K_F indicates less adsorption. This is in agreement with the difference in Freundlich parameter $1/n$, which indicates the degree of nonlinearity in an isotherm. The adsorption isotherms in different soils were characterized as per the Giles classification (37). In Ashoknagar soils, the isotherm was almost linear. In Pune soils, the "L" type isotherm was observed ($1/n < 1$) indicating a higher affinity of TFS to the adsorbing sites at low concentration. There was a reducing trend in the extent of adsorption against the increase in TFS concentration. In Monheim soils, a "C" type adsorption isotherm was observed, which reflects low adsorption at low initial concentration. Furthermore, it indicates a constant partitioning of TFS between the soil particles and the soil solution with newer adsorption sites becoming available as the initial concentration increases. The same explanation holds good for the adsorption of TFS-acid with $1/n > 1$.

Desorption. Desorption of TFS increased as Pune < Ashoknagar < Monheim, which is opposite to the order with which the extent of adsorption varied among the test

Table 8. Adsorption–Desorption of TFS and TFS-Acid in Soils

soil	TFS					TFS-acid				
	amount applied (μg)	total adsorbed (μg)	% adsorbed	amount desorbed (μg)	adsorbed amount desorbed (%)	amount applied (μg)	total adsorbed (μg)	% adsorbed	amount desorbed (μg)	adsorbed amount desorbed (%)
Ashoknagar	304.5	240.3	78.9	20.4	8.5	304.5	150.5	49.4	97.0	82.7
	202.8	150.5	74.2	17.2	11.4	202.8	91.9	45.3	73.0	87.9
	164.7	124.5	75.6	15.5	12.4	164.7	74.1	44.9	35.8	76.6
	18.2	14.6	80.2	1.7	11.6	8.2	1.7	20.7	1.36	26.5
	4.1	3.2	78.1	0.42	13.1	4.1	1.0	24.4	0.4	24.0
Pune	304.5	245.1	80.5	19.5	7.9	304.5	156.0	51.2	76.6	62.2
	202.8	153.1	75.5	18.0	11.8	202.8	97.3	47.9	62.9	71.2
	164.7	126.6	76.9	12.8	10.1	164.7	82.2	49.9	32.2	45.7
	18.2	15.7	86.3	1.4	8.9	8.2	1.8	21.9	1.14	75.6
	4.1	3.5	85.4	0.3	8.6	4.1	0.47	11.5	0.04	85.1
Monheim	304.5	232.2	76.3	27.2	11.7	304.5	128.7	42.3	124.4	59.5
	202.8	145.5	71.8	21.0	14.4	202.8	83.5	41.2	88.3	67.1
	164.7	119.1	72.3	19.7	16.5	164.7	68.1	41.4	73.8	47.3
	18.2	9.2	50.6	1.8	19.6	8.2	2.5	30.5	4.5	45.6
	4.1	1.7	41.5	0.4	23.5	4.1	0.2	5.1	0.6	95.2

Table 9. Adsorption Parameters of TFS and TFS-Acid in Different Soils

soil	sorption model	parameter	before desorption		after desorption	
			TFS	TFS-acid	TFS	TFS-acid
Ashoknagar	Freundlich	K_F	9.88 ± 0.23	1.76 ± 0.11	16.94 ± 0.26	0.82 ± 0.01
		$1/n$	1.00 ± 0.03	1.33 ± 0.05	1.08 ± 0.01	0.515 ± 0.004
		n	1.00 ± 0.03	0.75 ± 0.03	0.93 ± 0.01	1.94 ± 0.01
		R^2	0.99	0.99	0.99	0.97
	Langmuir	$1/K_1$	-0.04 ± 0.02	0.06 ± 0.17	-0.05 ± 0.02	0.65 ± 0.08
		K_1	-25.2 ± 10.9	16.8 ± 48.8	-18.7 ± 5.7	1.5 ± 0.2
		$1/K_1K_2$	0.11 ± 0.01	1.02 ± 0.13	0.08 ± 0.01	0.16 ± 0.01
		K_2	-0.36 ± 0.19	0.06 ± 0.16	-0.65 ± 0.25	4.20 ± 0.18
		R^2	0.99	0.99	0.99	0.98
	linear	K_D	10.16 ± 0.24	2.82 ± 0.01	18.41 ± 0.35	0.37 ± 0.01
		K_{OC}	267.4 ± 6.3	74.3 ± 0.3	484.6 ± 9.2	9.7 ± 0.3
		K_{OM}	155.1 ± 3.6	43.1 ± 0.2	281.1 ± 5.4	5.6 ± 0.2
		R^2	0.97	0.98	0.92	0.87
	Pune	Freundlich	K_F	11.38 ± 0.23	1.40 ± 0.13	18.27 ± 0.19
$1/n$			0.88 ± 0.02	1.58 ± 0.08	0.99 ± 0.03	1.35 ± 0.14
n			1.13 ± 0.03	0.63 ± 0.03	1.01 ± 0.03	0.74 ± 0.08
R^2			0.99	0.99	0.99	0.98
Langmuir		$1/K_1$	-0.005 ± 0.019	-1.81 ± 0.84	-0.003 ± 0.025	-27.52 ± 29.78
		K_1	-221 ± 923	-0.55 ± 0.26	-315 ± 2445	-0.04 ± 0.04
		$1/K_1K_2$	0.06 ± 0.01	3.16 ± 1.13	0.05 ± 0.01	10.92 ± 10.39
		K_2	-0.07 ± 0.31	-0.57 ± 0.47	-0.06 ± 0.46	-2.52 ± 5.13
		R^2	0.99	0.91	0.99	0.98
linear		K_D	10.91 ± 0.16	3.08 ± 0.02	19.33 ± 0.03	1.08 ± 0.01
		K_{OC}	198.4 ± 2.8	56.0 ± 0.5	351.5 ± 0.5	19.6 ± 0.2
		K_{OM}	115.1 ± 1.6	32.5 ± 0.3	203.9 ± 0.3	11.4 ± 0.1
		R^2	0.96	0.99	0.91	0.77
Monheim		Freundlich	K_F	5.84 ± 0.20	1.06 ± 0.05	12.07 ± 0.15
	$1/n$		1.47 ± 0.01	1.49 ± 0.03	1.24 ± 0.07	1.12 ± 0.06
	n		0.68 ± 0.01	0.67 ± 0.01	0.81 ± 0.05	0.89 ± 0.05
	R^2		0.99	0.94	0.99	0.95
	Langmuir	$1/K_1$	-0.30 ± 0.08	-2.68 ± 0.88	-0.30 ± 0.16	-8.77 ± 5.28
		K_1	-3.37 ± 0.95	-0.37 ± 0.12	-3.32 ± 1.76	-0.11 ± 0.07
		$1/K_1K_2$	0.53 ± 0.05	4.41 ± 1.05	0.22 ± 0.06	2.64 ± 1.29
		K_2	-0.56 ± 0.21	-0.61 ± 0.35	-1.35 ± 1.09	-3.32 ± 3.61
		R^2	0.99	0.71	0.99	0.98
	linear	K_D	9.28 ± 0.22	2.17 ± 0.02	13.72 ± 0.14	1.26 ± 0.07
		K_{OC}	549.4 ± 12.8	128.6 ± 1.3	811.8 ± 8.2	74.6 ± 4.0
		K_{OM}	318.7 ± 7.4	74.6 ± 0.7	470.9 ± 4.7	43.2 ± 2.3
		R^2	0.97	0.99	0.94	0.86

soils (**Table 8**). In Ashoknagar soils, desorption varied between 8.5 and 13.1%, whereas in Pune soils, 8.0–11.8% of the adsorbed TFS got desorbed. An overall low desorption indicates a firm retention of TFS on the soils. In the case of TFS-acid, however, desorption was high, indicating a loose retention on the soil surface.

Desorption of TFS and TFS-acid conformed to the Freundlich and Langmuir isotherms in all three soils (**Table 9**). The K_D values pertaining to desorption were consistently higher than the corresponding values for adsorption. Sukul and Spittler noted similar observations while investigating the adsorption–desorption of the fungicide metalaxyl in soils (38). Such a

phenomenon might have occurred due to a possible hysteresis effect during desorption involving various forces that caused higher retention of TFS molecules after desorption than that of adsorption at unit equilibrium concentration. Furthermore, such observations indicate that the process of adsorption was reversible.

Conclusion. The pesticide TFS degraded following first + first-order kinetics with a half-life of less than 3 days in three different test soils from different geographic origins. Hydrolysis was the major pathway of degradation resulting in TFS-acid as the major identified metabolite. TFS was strongly adsorbed on all of the test soils indicating its strong interaction with the binding sites, namely, organic and clay fractions of the soils. The results of the degradation and adsorption-desorption studies indicate that TFS does not pose any appreciable risk to overall soil health; thus, the risk of contamination of groundwater and the next crop is also not a point of concern. Experimental and theoretical calculations prove that TFS is effectively degraded during photolysis irrespective of geographic latitudes.

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