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Investigation of the Fate of Trifluralin in Shrimp

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ABSTRACT: Juvenile Pacific white shrimp (*Litopenaeus vannamei*) were exposed to trifluralin at 0.1 and 0.01 mg L^{-1} for 72 h under controlled conditions. Samples of shrimp and tank water were collected at intervals up to 48 days after exposure. Analysis of the shrimp tissues by gas chromatography−mass spectrometry (GC-MS) and ultrahigh-performance liquid chromatography−quadrupole time-of-flight mass spectrometry (UHPLC-qToF-MS) in combination with profiling and metabolite identification software (Agilent MET-ID and Mass Profiler Professional) detected the presence of parent trifluralin together with two main transformation products (TPs), 2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole (TP1) and 2-amino-6-nitro-4-(trifluoromethyl)phenyl)propylamine (TP2). The highest concentration of trifluralin, determined by GC-MS, was 120 μg kg^{-1} at 0 day withdrawal. Residues of trifluralin (CCα = 0.25 μg kg^{-1}, CCβ = 0.42 μg kg^{-1}) were detectable for up to 7 days after exposure. Similarly, the highest concentrations of TP1 and TP 2, determined by liquid chromatography−mass spectrometry/mass spectrometry (LC-MS/MS), were 14 and 18 μg kg^{-1}, respectively. Residues of TP1 (CCα = 0.05 μg kg^{-1}, CCβ = 0.09 μg kg^{-1}) and TP2 (CCα = 0.1 μg kg^{-1}, CCβ = 0.17 μg kg^{-1}) were detectable for up to 4 and 24 withdrawal days, respectively.

KEYWORDS: *trifluralin, shrimp, metabolism, aquaculture, profiling, photolysis*

INTRODUCTION

Trifluralin, a dinitroaniline compound, is widely used as a presowing/pre-emergence herbicide. The use of trifluralin has been banned in the European Union (EU) since 2007 (Commission Decision 2007/629/EC)[1] due to insufficient data on aquatic risk assessment, toxicity of certain metabolites and consumer exposure for noncereal applications.[2] Nevertheless, trifluralin is still widely use in other parts of the world (e.g., United States and Asia). In fact, trifluralin was one of a number of candidate compounds that have been screened for efficacy against pathogenic aquatic fungi and, hence, as a possible replacement for malachite green, a potential carcinogen.[3] Trifluralin is virtually nontoxic to mammals but can be highly toxic to aquatic organisms.[4] When banana shrimp (*Penaeus merguiensis*) were exposed to trifluralin, the median lethal concentrations (LC_{50}) at post larval (PL) stages PL10, PL20, and PL30 were 7.97, 9.98, and 13.13 mg L^{-1}, respectively.[3] However, the survival rate of *P. merguiensis* PL5 exposed to lower concentrations of trifluralin (0.5−1 mg L^{-1}) was significantly higher than that of the nonexposed control group, an observation attributed to the control of pathogenic fungi.[3] Trifluralin pesticide formulations have also been reported to treat larval mycosis in experimental studies[5−7] and as a treatment in shrimp farming.[8] It is not surprising, therefore, that batches of shrimp traded from Vietnam to Japan in 2010 were found to contain trifluralin at concentrations above the Japanese maximum residue limit (MRL) of 1 μg kg^{-1} (Japan Food Chemical Research Foundation).[9,10] In response, the Vietnamese Ministry of Agriculture and Rural Development (MARD) issued Decision No. 2985 requiring increased monitoring of consignments of shrimp and *Pangasius* sp. (catfish) exported to Japan.[9] Subsequent awareness within the EU has resulted in increased monitoring activity. This led to 10 EU rapid alerts[11] for trifluralin residues (35−204 μg kg^{-1}) in *Pangasius* imported into the EU during 2011 but none in shrimp. EU Commission Regulation No. 600/2010 specifies an MRL of 10 μg kg^{-1} trifluralin in terrestrial animal products.[12]

Because trifluralin is known to undergo extensive photodecomposition in aqueous media,[13] it is possible that the parent compound might not be the most appropriate marker of residue for detecting use. Also, the metabolism of trifluralin is well studied in mammalian species,[14] but there is limited information on the uptake, depuration, and metabolism of trifluralin for aquatic organisms and, to the best of our knowledge, none on shrimp. In one such experiment, freshwater invertebrates (*Lumbriculus variegates*, *Sphaerium corneum*, and *Chrionomus riparius*) were exposed to ^{14}C-labeled trifluralin for 48 h. HPLC radiochromatograms of *L. variegates* and *S. corneum* showed peaks only for parent trifluralin, indicating no metabolism.[15] By contrast, radiochromatograms of *C. riparius* from the same study show peaks for trifluralin along with two additional early-eluting peaks with total radioactivities of 17.5, 25.1, and 1.3%, respectively.[14] Unfortunately, the structures of the additional peaks and possible transformation products were not identified.

In this paper we report, for the first time, a study on the fate of trifluralin in shrimp. A controlled study on the exposure of shrimp to trifluralin was undertaken with the objective of detecting and identifying any transformation products (TPs)

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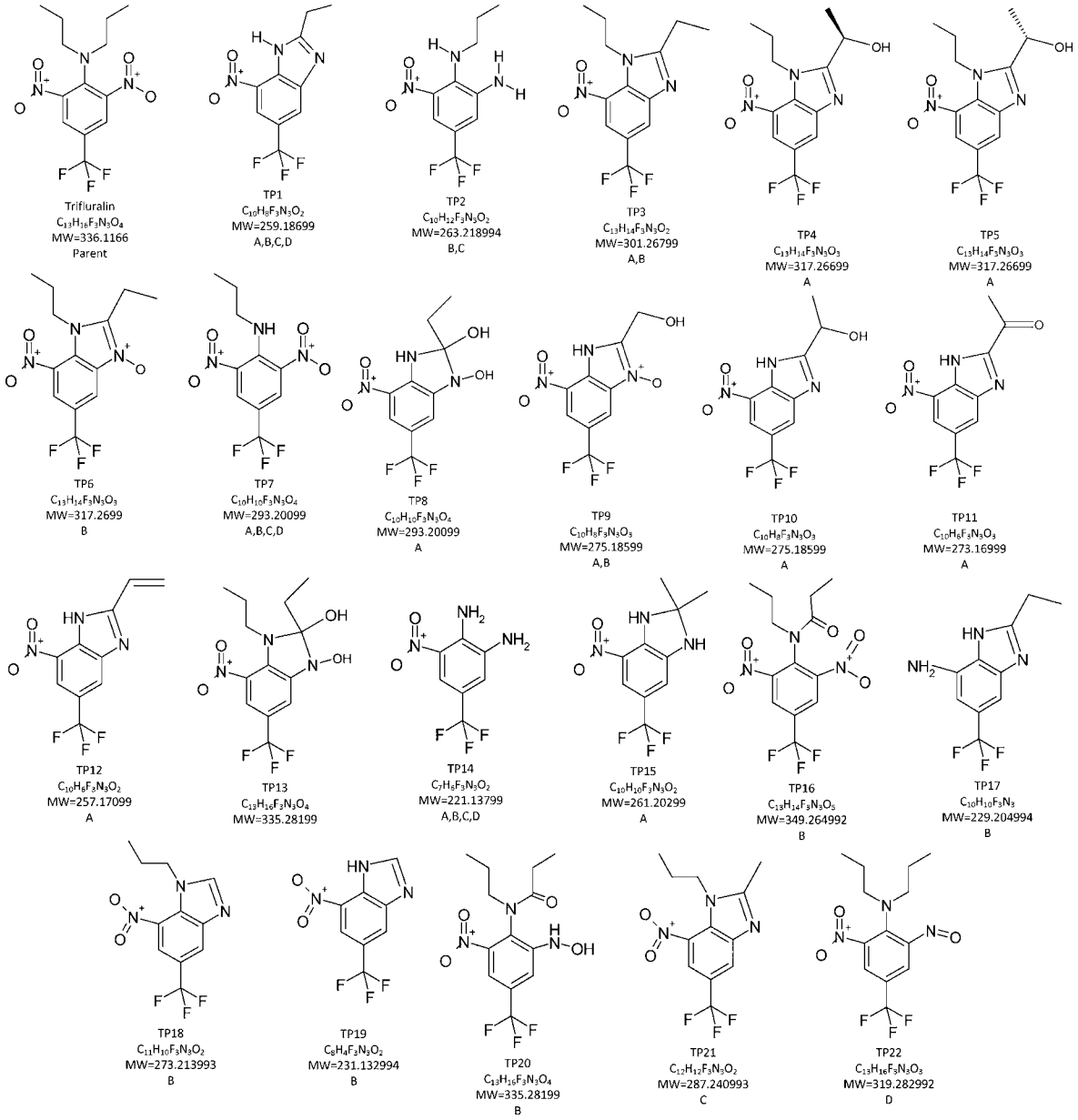

Figure 1. Transformation products from UV photolysis of a saturated seawater solution of trifluralin: products resulting from (A) photolysis, (B) soil metabolism, (C) mammalian metabolism, and (D) microbial metabolism.

that might be more appropriate (than parent trifluralin) to detect trifluralin use in shrimp production.

MATERIALS AND METHODS

Standards and Reagents. Trifluralin was purchased from Sigma (Dorset, UK). Deuterated trifluralin-d_{14} was purchased from QMX Laboratories (Thaxted, UK). 2-Ethyl-7-nitro-5-(trifluoromethyl)-benzimidazole (TP1) and 2-amino-6-nitro-4-(trifluoromethyl)phenyl)-propylamine (TP2) (Figure 1) were custom synthesized by Biomedical Research Centre, Sheffield Hallam University, UK. ^{1}H NMR (400 MHz, $CDCl_3$) for TP1: δ 10.57 (br s, NH), 8.40 (s, Ph), 8.29 (s, Ph), 3.1 (q, CH_2), 1.53 (t, CH_3). ^{1}H NMR (400 MHz, $CDCl_3$) for TP2: δ 7.75 (s, Ph), 7.05 (s, Ph), 6.4 (br m, NH), 5.10 (br s, NH_2), 3.15 (q, CH_2), 1.6 (m, CH_2), 0.95 (t, CH_3). Bondesil-PSA and C18 were purchased from Agilent Technologies (Wokingham, UK). Magnesium sulfate (anhydrous) was purchased from Alfa Aesar (Heysham, UK). Sodium chloride and HPLC grade solvents were purchased from Fisher Scientific (Loughborough, UK). Treflan at 480 g L^{-1} was donated by Dow Chemicals, UK.

Chemical Modeling. A comprehensive literature search was undertaken to collate available information (chemical structures, properties, etc.) on the metabolites and degradation products of trifluralin. In addition, computer modeling programs BiotS (biotransformation susceptibility),[16] BBD-PPS (biocatalysis/biodegradation database pathway prediction system),[17] and Meteor version 12.0 (Lhasa Limited) (using processing constraints; plausible, n = 2, do not grow from phase II products) were employed to predict the structures of possible transformation products.

Photodegradation of Trifluralin in Seawater. Trifluralin has limited solubility in water (approximately 0.2−0.4 mg L^{-1}).[18] To maximize the concentration of photodegradation products, a saturated solution of trifluralin (nominal concentration of 5 mg L^{-1}) in seawater (salinity at 35 parts per thousand) was exposed to UV radiation (24 W, 365 nm tubes) for 24 h. A separate saturated solution was left exposed to ambient daylight conditions for up to 4 days. To avoid fouling the mass spectrometer by direct injection of concentrated salt solutions, 5 mL seawater samples were added to 5 mL of acetonitrile and 5 g of magnesium sulfate and vortex mixed. The upper acetonitrile layer was analyzed by UHPLC-qTOF-MS (see below).

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Exposure of Shrimp to Trifluralin. Juvenile Pacific white shrimp (*Litopenaeus vannamei*) at 0.5−5 g body weight were held in five 900 L tanks (A−E) containing seawater (salinity at 35 parts per thousand) at 26 °C with a stocking density of 100 shrimps per tank. The tanks were assigned to one of three groups. Group 1 (tanks A and B) and group 2 (tanks C and D) were employed for exposure to trifluralin at 0.1 and 0.01 mg L^{-1}, respectively. Group 3 (tank E) was designated a control. Shrimps were fed via an automatic feeder to allow constant feeding for 20−22 h with Vannamei diet (1.5−1.8 mm pellets, Dragon's Feed, UK). To ensure that feed was distributed throughout the tank and avoid territories being formed by larger or more dominant shrimp, the positions of the automatic feeders were rotated and an additional manual feed was offered three times each day. An artificial lighting regimen was employed, providing 200−400 lx of luminance.

A pesticide formulation (Treflan at 480 g L^{-1} trifluralin) was used for shrimp exposure instead of pure trifluralin in an attempt to reflect likely shrimp production practices. Tank water (300 L, 33% of the total volume) was drained at 24 and 48 h and replenished with water containing Treflan to maintain a nominal trifluralin concentration of either 0.1 or 0.01 mg L^{-1}. This procedure was carried out to offset any loss of trifluralin from adsorption onto the tank surfaces (see below). The tanks were maintained essentially at static conditions during the exposure phase. The normal flow (1−3 L min^{-1}) was resumed 72 h after trifluralin exposure. Shrimp samples were collected from control and exposure tanks at 0, 0.25, 1, 2, 4, 7, 24, and 48 days after exposure. The typical body weight of individual shrimps analyzed from days 24 and 48 (post exposure) was approximately 10 g, of which approximately 50% was muscle tissue. Shrimp samples were stored at −80 °C after sampling until analysis. Tank water samples and waste material were also collected for analysis.

Sample Extraction and Cleanup. Whole shrimps were shelled before extraction. When the muscle mass was below the required amount for analysis (5 g), multiple shrimps were pooled to provide sufficient sample mass. Samples of shrimp (muscle) were extracted using a generic acetonitrile extraction method based on the QuEChERS method.[19] Muscle samples (5 g) were homogenized in acetonitrile/water (10 mL, 10:4 v/v), followed by vortex mixing with sodium chloride (1 g) and magnesium sulfate (4 g), and centrifuged (3600*g* at 5 °C, 15 min). The acetonitrile supernatant (7 mL) was cleaned up using dispersive C18 + PSA (250 mg of each) and dried with magnesium sulfate (1 g). Initial analysis was carried out using UHPLC-qToF-MS to detect TPs of trifluralin in shrimp muscle. GC-MS and LC-MS/MS were used for subsequent quantification of parent trifluralin and TPs, respectively.

UHPLC-qToF-MS Analysis: Profiling and Identification of Transformation Products. Analysis was carried out using an Agilent 6530 Accurate-Mass Q-TOF coupled to an Agilent 1290 Infinity LC/autosampler. Chromatographic separation was performed using an Acquity HSS T3 UPLC column (2.1 × 50 mm, 1.8 μm), with a mobile phase gradient composed of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). A linear gradient of 95 to 1% A (v/v) was used over 0−15 min. The injection volume was 5 μL, and the flow rate was set at 0.2 mL min^{-1}. Analysis was performed in high-resolution mode (4 GHz) using both electrospray positive and negative with a mass range of m/z 50−1700. Hexakis(2,2,3,3-tetrafluoropropoxy)-phosphazine was used as a lock mass throughout the analysis. Data processing and metabolite profiling were carried out using a suite of software programs [Agilent MassHunter (vB 03.01), Mass Profiler (vB 02.00), and Metabolite ID (vB 02.00)].

Trifluralin Adsorption Experiments. To assess if trifluralin could be adsorbed onto the tank surfaces (glass reinforced plastic) in an aqueous environment, a 0.2 μg mL^{-1} aqueous solution of trifluralin held in a plastic (polypropylene) vial was repeatedly injected into a LC-ToF system. After 1 h, no signal was detected for trifluralin. However, upon exchange of the aqueous solvent with acetonitrile, the trifluralin signal slowly reappeared over the course of several hours, indicating that trifluralin had been adsorbed onto the vial wall under an aqueous environment.

GC-MS Analysis: Quantification of Trifluralin. Trifluralin was quantified using an Agilent 6890A GC coupled to an Agilent 5973 MSD. Separation was performed using a Varian VF5-MS GC column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, 10 m EZ-guard). The programmed temperature vaporization (PTV) injection volume was 10 μL with an injection speed of 10 μL s^{-1}, a solvent vent for 15 s (50 mL min^{-1}, 8.2 psi), and spiltless sample transfer (purge flow, 50 mL min^{-1} at 150 s). The PTV initial temperature was set at 60 °C, ramped to 250 °C at 12 °C min^{-1}, and then held at 250 °C for 2 min. The GC oven temperature was set at 60 °C for 2 min and then ramped to 160 °C at 25 °C min^{-1}, to 200 °C at 4 °C min^{-1}, to 290 °C at 10 °C min^{-1}, and then held at 290 °C for 5 min. Analysis was performed using electron impact (EI) in selected ion monitoring (SIM) mode. Ions monitored included m/z 335 (parent), 306, 290, and 264, with m/z 306 yielding the highest detector response.

In addition, analysis by GC negative chemical ionization (NCI) MS was also employed to provide further evidence of the identity of trifluralin detected by GC-EI-MS. GC-NCI-MS was performed using a Varian 3800 GC coupled to a Varian 1200 mass spectrometer. Separation was achieved using a Zebron ZB50 GC column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The spectrometer was operated in SIM mode using negative chemical ionization with methane as CI gas. The ions monitored were m/z 335 (parent ion), 336, 337, and 305. Deuterated trifluralin (d_{14}) was used as an internal standard (monitored at m/z 315) and added to samples prior to extraction.

LC-MS/MS: Quantification of Transformation Products. TP1 and TP2 were quantified using an Alliance 2695 HPLC system coupled to a Micromass Quattro Ultima Pt triple-quadrupole instrument (Waters, Elstree, UK). Separation was performed using a Synergi Fusion-RP HPLC column (100 × 2.00 mm, 2.5 μm) (Phenonmenex, Macclesfield, UK) with a water (C) and methanol (D) gradient. The gradient was 0−1 min, 95−50% (A); 1−5 min, 50−1% (A); 5−9 min, 1% (A); 9.1 min, 95% (A). The injection volume was 5 μL. Source and desolvation temperatures were set at 120 and 300 °C, respectively. Capillary and cone voltages were set at 2.8 kV and 35 V, respectively. Desolvation and cone gas flow rates were set at 700 and 100 L h^{-1}, respectively. Analyses were performed using negative electrospray ionization in multiple reaction monitoring (MRM) mode. MRM transitions for TP1 were m/z 258 → 212 and 258 → 227 and for TP2 were m/z 262 → 204 and 262 → 215. Collision energies were set at 25 and 10 eV for TP1 and TP2, respectively, with a dwell time of 0.1 s for all MRM transitions.

Method Validation: CCα/CCβ Values and Stability. The CCα (decision limit) and CCβ (detection capability) values were calculated for trifluralin, TP1, and TP2 as outlined in Commission Decision 2002/657/EC.[20] Using this approach, shrimp muscles were spiked with parent trifluralin at 0.5, 0.75, and 1.0 μg kg^{-1}; TP1 and TP2 were spiked at 0.2, 0.3, and 0.4 μg kg^{-1} in each of three batches (n = 7 per spiking concentration per batch, i.e., n = 21 per batch). Each sample extract was analyzed by GC-MS (trifluralin) and by LC-MS/MS (TP1 and TP2). The three individual batches were analyzed on three separate days. CCα and CCβ values were then calculated using the calibration curve procedure (ISO 11843).[21]

To assess the storage stability of trifluralin, TP1, and TP2, muscle tissue samples spiked with each compound (5 μg kg^{-1}) were stored at −20 °C over a 4 week period. Sample extracts of the spikes (5 μg kg^{-1}) were also stored at 4 °C and analyzed over a 4 week period.

RESULTS AND DISCUSSION

UHPLC-qToF-MS Analysis of Seawater Containing Trifluralin. The exact monoisotopic masses, corresponding to 52 potential degradation products identified by chemical modeling,[22] were used to reverse search the ToF-MS data. A total of 17 unique accurate mass ions were detected in the UV photolyzed seawater saturated with trifluralin. The accurate masses correlate to 22 possible TPs (Figure 1). No additional ion masses were detected by application of mass profiling software (Agilent Mass Profiler Pro and Met-ID) to the raw ToF-MS data. A reduced number of mass ions were identified for the trifluralin solution exposed to sunlight. The majority of

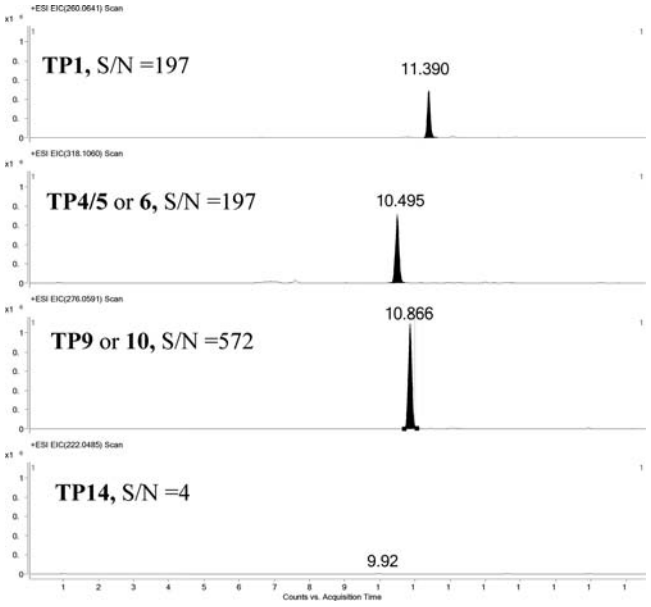

Figure 2. LC-ToF (ESI+) chromatogram of TPs found in UV photolyzed trifluralin seawater.

the TPs were formed as a result of cyclization, N-dealkyllation, and/or nitroreduction of parent trifluralin under photolysis conditions. Some of the TPs have also been reported as a result of metabolism in soil or in mammalian species.[23]

The most responsive accurate mass ions observed under both photolysis conditions (UV and ambient daylight) corresponded to the assigned TP1, TP4/5 (isomers), TP6, TP9, and TP10 (Figure 2). TP1 and TP14 have been reported previously as

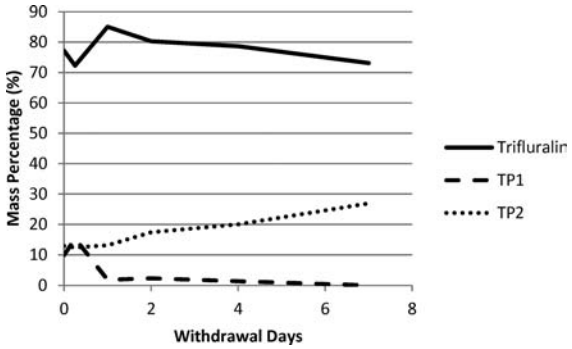

Figure 3. Mass percentage (total residues) of trifluralin, TP1, and TP2 for group 1 shrimp during the first 7 withdrawal days.

major photodegradation products in sterile buffer solution under artificial sunlight.[24] However, TP14 was detected in this study but was found not to be the most responsive ion with a signal-to-noise ratio (S/N) of 4 compared to TP1 with a S/N of 197 (Figure 2).

Initial LC-ToF analysis of the tank water samples detected only TP1 as a transformation product. Trifluralin was detectable at a concentration of 0.4 μg L^{-1} for up to 4 days after exposure using GC-MS analysis (group 1). Quantification of the main TPs and trifluralin in the tank water is discussed below.

UHPLC-qToF-MS Analysis of Muscle Tissue from Shrimp Exposed to Trifluralin. Initial UHPLC-qToF-MS analysis of 0 day withdrawal shrimp muscle samples from group 1 (0.1 mg L^{-1} exposure) showed that parent trifluralin was the most responsive marker residue 72 h after exposure to Treflan. Application of mass profiling software (Agilent Mass Profiler

Table 1. Summary of Validation Data Obtained for Trifluralin, TP1, and TP2 in Shrimp Muscle Tissue (n = 21)

	target concn (μg kg^{-1})	CCα (μg kg^{-1})	CCβ (μg kg^{-1})	correl coeff (R^2)	recovery (%)	CV (%)
trifluralin	0.5	0.25	0.42	0.997–0.999	80[a]	14.8
TP1	0.2	0.05	0.09	0.977–0.997	98	5.5
TP2	0.2	0.1	0.17	0.980–0.993	109	11.2

[a]Apparent internal recovery (internal standard used).

Table 2. Concentrations (Micrograms per Kilogram) of Trifluralin in Shrimp Muscle after Exposure of Shrimp to Trifluralin at 0.1 mg L^{-1} (Group 1), 0.01 mg L^{-1} (Group 2), and 0 mg mL^{-1} (Group 3 = Control)

			withdrawal days							
analyte (n = 1)	group	tank	0	0.25	1	2	4	7	24	48
trifluralin	1	A	120	37	26	5.1	5.1	0.9	<0.25	<0.25
		B	90	13	15	4.9	2.6	1.0	<0.25	<0.25
	2	C	15	4.6	0.7	0.9	0.4	<0.25	<0.25	<0.25
		D	15	3.1	1.7	0.8	0.7	<0.25	<0.25	<0.25
	3	E	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
TP1	1	A	7.0	3.6	1.4	0.2	0.1	<0.1	<0.1	<0.1
		B	14	5.4	0.4	0.1	0.1	<0.1	<0.1	<0.1
	2	C	0.8	0.4	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
		D	1.1	0.9	0.29	<0.1	<0.1	<0.1	<0.1	<0.1
	3	E	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
TP2	1	A	14	8.3	6.4	1.0	1.1	0.4	0.1	<0.1
		B	18	4.3	3.2	1.1	1.0	0.3	0.1	<0.1
	2	C	3.3	1.4	0.3	0.2	0.1	<0.1	<0.1	<0.1
		D	1.7	1.3	0.5	0.2	0.2	<0.1	<0.1	<0.1
	3	E	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

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Table 3. Tank Water Data (Nanograms per Milliliter) from Group 1[a]

		withdrawal days								
analyte (n = 5)	tank	−3	−2[b]	−2[c]	−1[b]	−1[c]	0	0.25	1	2	4	7
trifluralin	A	130 (5)	7.6 (6)	27.5 (7)	2.7 (5)	21.6 (6)	2.6 (6)	1.1 (7)	0.7 (7)	0.6 (8)	<0.25	<0.25
	B	40 (7)	10.5 (9)	32.9 (6)	3.1 (6)	18.3 (5)	2.9 (8)	1.4 (8)	0.9 (7)	0.6 (8)	0.5 (9)	<0.25
TP1	A	0.07 (7)	0.47 (8)	0.32 (7)	0.46 (6)	0.34 (6)	0.51 (9)	0.14 (9)	0.01 (8)	<0.01	<0.01	<0.01
	B	0.03 (10)	0.16 (9)	0.12 (6)	0.27 (8)	0.21 (8)	0.31 (7)	0.11 (9)	0.03 (12)	<0.01	<0.01	<0.01
TP2	A	<0.01	0.06 (9)	0.05 (12)	0.05 (10)	0.03 (12)	0.07 (8)	<0.01	<0.01	<0.01	<0.01	<0.01
	B	<0.01	<0.01	<0.01	<0.01	0.02 (10)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Relative standard deviation (%, n = 5) is given in parentheses. [b]Values before tank refill with fresh trifluralin solution. [c]Values after tank refill with fresh trifluralin solution at 0.1 mg L^{-1} at 30% total volume.

Table 4. Analysis of Catfish and Shrimp from Southeast Asia (n = 1)

sample type	origin	trifluralin (μg kg^{-1})	TP1 (μg kg^{-1})	TP2 (μg kg^{-1})
catfish	Vietnam	0.26	<0.1	0.2
catfish	Thailand	<0.25	<0.1	<0.1
catfish	Vietnam	<0.25	<0.1	<0.1
catfish	Vietnam	2.04	<0.1	0.1
catfish	Thailand	<0.25	<0.1	<0.1
catfish	Vietnam	3.17	<0.1	0.1
catfish	Thailand	<0.25	<0.1	<0.1
shrimp	Vietnam	<0.25	<0.1	0.1
shrimp	Thailand	<0.25	<0.1	<0.1
shrimp	Vietnam	<0.25	<0.1	<0.1
shrimp	Vietnam	<0.25	<0.1	<0.1

Pro and Met-ID), as well as reverse searching of the ToF-MS raw data, revealed TP1 and TP2 as the main transformation products present in shrimp muscle. Interestingly, TP2 was not detected in seawater subjected to daylight photolysis conditions or in the Treflan-treated tank water. By contrast, compound TP1 was detected in both UV and daylight photolysis conditions and the Treflan-treated tank water. TP1 and TP2 have been reported previously as metabolites in soil systems and rat urine.[13,25] Standards of TP1 and TP2 were therefore custom synthesized to allow identification and quantification in shrimp muscle samples.

Validation. The results of the validation of a quantitative method, according to Commission Decision 2002/657/EC, for trifluralin, TP1, and TP2 in shrimp muscle are presented in Table 1. CCα and CCβ were calculated using the calibration curve approach according to ISO11843. All analyte recoveries are in the range of 80−110% and are within the acceptable range of 50−120% recovery for analyte concentration of <1 μg kg^{-1} as outlined in Commission Decision 2002/657/EC. Coefficients of variance (CV) of <20% were calculated from the validation data for the three analytes, satisfying the precision requirements of analysis at <100 μg kg^{-1}.[26] No sign of analyte degradation was observed in the spiked shrimp muscle (−20 °C) or sample extracts (4 °C) over a 4 week period.

Quantification of Trifluralin, TP1, and TP2 in Shrimp Muscle. The concentration of residues in the muscle tissue of exposed shrimp and in control shrimp are summarized in Table 2. The majority of available samples were used for profiling analysis. Because of the limited number of trifluralin-treated shrimp samples available after profiling analysis, it was possible to perform quantification of only one sample per time point; thus, statistical treatment of the results was not possible.

For the highest exposure concentration (0.1 mg L^{-1} trifluralin, group 1), trifluralin is detectable at 1.0−0.9 μg kg^{-1} for up to 7 days after exposure. TP1 and TP2 were detectable at 0.1 μg kg^{-1} for up to 4 and 24 days after exposure, respectively.

Figure 3 shows the combined group 1 mass percentage (total residues) of trifluralin, TP1, and TP2 in shrimp tissue muscle to be approximately 77, 10, and 13%, respectively, at 0 withdrawal days. The mass percentages of trifluralin and TP1 reduce to 73 and 0%, respectively, 7 days after exposure, whereas TP2 increases to 27%. This clearly indicates trifluralin is the major marker for detection in shrimp muscle. The extended period of detection for TP2 (CCα = 0.1 μg kg^{-1}) of up to 24 withdrawal days is mainly due to the lower detection limit compared to

that of trifluralin (CCα = 0.25 μg kg^{-1}). The decrease in the concentration (dilution) of trifluralin and TPs, with increase in tissue mass, is a factor that needs to be considered when reporting limits are set.

Formation of Transformation Products. Tank water samples from group 1 were reanalyzed using the validated GC-MS (trifluralin) and LC-MS/MS (TP1 and TP2) methods (Table 3). During the 3 day exposure period (−3 to 0 withdrawal days), the concentration of trifluralin can be observed to decrease and then increase at 24 h intervals due to the replenishment of fresh trifluralin at −1 and −2 withdrawal days. The rapid decrease in concentration of trifluralin in the water is probably attributable to adsorption onto the tank surfaces and possibly photodegradation. Fresh seawater flow at 1−3 L min^{-1} was resumed in the tank at 0 day withdrawal. Samples taken after this point showed that residues of parent trifluralin can persist in the tank system for up to 4 days after exposure. Because trifluralin is adsorbed onto the tank surfaces, it is possible that this chemical was partitioning back into the flowing fresh seawater until it had totally depleted. TP1 was eliminated from the tank system just over 24 h after fresh seawater flow had resumed to the tank. TP2 was not detected in seawater samples taken shortly after the flow was restarted.

The accumulation of TP1 in shrimp muscle is possibly due to the uptake of TP1 present in the tank water. Formation of TP1 from the metabolism of trifluralin by the shrimp cannot be ruled out, although it is *unlikely* to represent a significant portion because the mass percentage of TP1 in shrimp tissue falls significantly from 12 to 2% after 1 day of withdrawal (Figure 3), in line with its elimination from the tank water. At this point, trifluralin still accounts for >70% of residues present in the shrimp (Table 3).

TP2 is a significant transformation product in the shrimp muscle but is barely detectable in the tank water. It is likely that it is formed either directly from the metabolism of trifluralin and/or TP1 (a photodegradation product found in the tank water) and not from uptake. Further work is needed to determine the formation mechanism of these transformation products.

Analysis of Survey Samples. Additional analysis was performed on a small number of catfish (7) and shrimp (4) samples originating from Southeast Asia. The results are summarized in Table 4. Three samples of catfish contained residues of both trifluralin and TP2, whereas one shrimp sample contained TP2 at 0.1 μg kg^{-1} in the absence of trifluralin. TP1 was not detected in any of the samples. All of the samples found to contain residues originated from Vietnam. The preliminary data show that TP2 is formed in both shrimp and catfish. UHPLC-qToF analysis of the catfish samples containing trifluralin and TP2 did not reveal any other TPs at detectable concentrations. A future study of catfish using a higher exposure concentration of trifluralin will be required to determine if other TPs (metabolites) occur in this species. The results from this limited survey provide evidence that trifluralin and its metabolite TP2 do occur in shrimp and catfish. The metabolite TP2 can be detected for extended periods after exposure to trifluralin and, thus, is a useful additional marker for the indicative use of trifluralin in shrimp production and possibly in other aquaculture products. A larger survey including other species is required to provide further supporting evidence.

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