Dietary Exposure to Bifenthrin and Fipronil Impacts Swimming Performance in Juvenile Chinook Salmon (Oncorhynchus tshawytscha)

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ABSTRACT: Two commonly used insecticides, bifenthrin and fipronil, can accumulate in the prey of juvenile Chinook salmon, yet the effects of dietary exposure are not understood. Therefore, to better characterize the effect of a dietary exposure route, juvenile Chinook salmon were fed chironomids dosed with a concentration of 9 or 900 ng/g of bifenthrin, fipronil, or their mixture for 25 days at concentrations previously measured in field-collected samples. Chinook were assessed for maximum swimming performance ($U_{\text{max}}$) using a short-duration constant acceleration test and biochemical responses related to energetic processes (glucose levels) and liver health (aspartate aminotransferase (AST) activity). Chinook exposed to bifenthrin and bifenthrin and fipronil mixtures had a significantly reduced swimming performance, although not when exposed to fipronil alone. The AST activity was significantly increased in bifenthrin and mixture treatments and glucose levels were increased in Chinook following a mixture treatment, although not when exposed to fipronil alone. These findings suggest that there are different metabolic processes between bifenthrin and fipronil following dietary uptake that may influence toxicity. The significant reductions in swimming performance and increased levels of biochemical processes involved in energetics and fish health could have implications for foraging activity and predator avoidance in wild fish at sensitive life stages.

KEYWORDS: Chinook salmon, pesticides, dietary uptake, swimming performance, biochemical assessment, energetics

1. INTRODUCTION
An increase in the detection of pyrethroid and phenylpyrazole pesticides in watersheds of the San Francisco Bay and Sacramento-San Joaquin Delta (Bay-Delta) in California (USA) has raised concern for native fish populations.1 Bifenthrin, a type I pyrethroid, and fipronil, a phenylpyrazole, are among the two most commonly detected insecticides in aquatic systems within California, with concentrations measured in the environment inducing adverse effects to nontarget aquatic organisms.2,3 In a monitoring study of over 500 urban surface water samples across California, fipronil was detected in 49%, with concentrations exceeding chronic toxicity benchmarks in 48%.3 Additionally, bifenthrin was detected in 77% of samples collected in the Bay-Delta4 and detected in 78% of collected sediment samples in the United States, Australia, and China.5

Bifenthrin and fipronil persist in the organic matter of sediments and have the potential to be taken up by benthic organisms.6−8 Studies on the effect of insecticides in salmonids are most commonly based on waterborne exposures, yet the diet is likely an important route of exposure contributing to toxicity, as insecticides have the potential to bioaccumulate.9 Consequently, dietary exposure of insecticides to fish through the consumption of contaminated prey items may represent an important route of exposure that remains poorly understood. Bioaccumulation studies of wild-caught fish have demonstrated accumulation of both bifenthrin10,11 and fipronil,12 although contributions from individual exposure routes (dietary and aqueous) have not been considered.

Bifenthrin alters the release of neurotransmitters by binding to voltage-gated sodium channels.13−16 Aqueous exposures to concentrations of ≤1.5 μg/L bifenthrin have been shown to induce neurotoxic and neuroendocrine effects in salmonids following treatment.17−23 Furthermore, in larval fathead minnows (Pimephales promelas), genes involved in neuro-
muscular and energetic function pathways were dysregulated following exposure to 350 ng/L bifenthrin. Bifenthrin significantly increased the level of aspartate aminotransferase (AST), a predominant biomarker for assessing liver injury due to necrosis, in common carp (Cyprinus carpio) treated with 5.75 μg/L of bifenthrin. Additionally, Velisek et al. noted that bifenthrin-exposed fish had significantly increased plasma glucose levels, which could result from stress to maintain increased energy demands in fish following pesticide treatment. The toxicity of bifenthrin to fish was predominately determined through the use of aqueous exposures, with no known studies conducted that assessed the effects of chronic, dietary exposure of bifenthrin to fish, which might serve as an important route of exposure.

The phenylpyrazole, fipronil, binds to γ aminobutyric acid (GABA) receptors, blocking GABA-gated chloride channels in the nervous system, which can lead to hyperexcitability, paralysis, and death in target organisms. Although fipronil tends to exhibit greater toxicity toward invertebrates, recent studies have demonstrated a similar binding capacity to GABA receptors in fish and insects, alike, suggesting that fipronil may also be highly toxic to fish. Furthermore, in fish, fipronil has been shown to be rapidly transformed into biodegradation products including fipronil sulfone, which has a similar toxic potential and may be more persistent than the parent compound. 

2. MATERIALS AND METHODS

2.1. Chemicals. Bifenthrin (100.0% purity) and fipronil (97.7% purity), for spiking midges, were obtained from AccuStandard (New Haven, CT). Dibromooctfluorophenyl (DBOFB, 99.3% purity) and decachlorobiphenyl (DCBP, 99.4% purity, 200 µg/mL in acetone) were obtained from Sigma-Aldrich (St. Louis, MO) and 2,3,4,4′,6,6′-hexachlorobiphenyl (PCB-168, 98.8% purity, 100 µg/mL in isoctane) was obtained from AccuStandard (New Haven, CT) and used for measuring recovery. All solvents were Optima grade (Fisher Scientific, Waltham, MA). Silica (60–200 mesh, grade 62), sea sand, sodium sulfate (ACS grade), acetic acid (ACS grade), and copper powder were obtained from Fisher Scientific (Gibbstown, NJ). Bifenthrin, pronil sulfone, which has a similar toxic potential and may be more persistent than the parent compound.

2.2. Juvenile Chinook Husbandry. Juvenile Chinook salmon were obtained from the Illinois Department of Natural Resources’ Jake Wolf Hatchery in March 2020 (Topeka, IL). Fish were maintained in recirculating systems containing dechlorinated, biofiltered municipal water and fed a pellet diet (BioVita Fry, Bio-Oregon, WA) ad libitum daily under a 12:12 h light/dark photoperiod. Water chemistry was maintained at 11 ± 1 °C, pH 6.8–7.0, and dissolved oxygen >7 mg/L throughout the acclimation period. All experiments were performed in accordance with Southern Illinois University Institutional Animal Care and Use Committee (IACUC), protocol number 17-027.

2.3. Experimental Design. Juvenile Chinook salmon (average fork length = 15.0 ± 1.56 cm, average weight = 27.6 ± 9.18 g) were transferred into individual 10 L polycarbonate tanks on one of three recirculating systems filled with dechlorinated, municipal water chilled to 11 ± 1 °C (n = 12 tanks per treatment). Treatments belonging to the same group were placed on the same recirculating system to prevent any chance of leaching of pesticide residues from either food or waste into the circulating water. Additionally, the water passed through a mechanical filter and activated carbon filter to remove waste and particulates from the water. There were 12 fish per treatment (control, bifenthrin, fipronil, binary mixture), with each fish placed into a separate, individual exposure tank (n = 12). After a 3-day acclimation period, Chinook were fed freeze-dried chironomid larvae dosed with either solvent control (acetone), bifenthrin, fipronil, or a binary mixture at a rate of approximately 1.5% body weight per day for 25 days. The experiment duration was based on juvenile Chinook residency times within the Bay-Delta prior to outmigration and based on previous dietary exposure studies with pyrethroids. The target concentrations were 9 and 900 ng/g (on a dry weight basis) for each compound individually and in binary mixtures, with 9 ng/g measured in known Chinook salmon dietary items from the Bay-Delta in 2019.
900 ng/g representing 2 orders of magnitude greater concentration to simulate a high exposure event following storm conditions. There were 12 replicates per exposure treatment at each concentration, and 900 ng/g. Unconsumed food was removed from tanks daily. Water chemistry parameters were assessed twice weekly throughout the experiment and water samples were collected from exposure tanks to measure potential pesticide residues leached from food items or waste.

As a positive control, juvenile Chinook were exposed to 30 μg/L chlordene (nominal) for 96 h, based on studies demonstrating effects on swimming performance in salmonids.32 Briefly, juvenile Chinook (n = 3 per tank) were transferred to one of four 38 L glass aquaria containing dechlorinated municipal water chilled at 11 °C. After a 48 h acclimation period, a 50% water change was conducted using a precalculated amount of a chlorpyrifos stock solution in acetone (60 mg/L). Water changes of 50% were conducted daily over the 96 h exposure period.

2.4. Dosing of Chironomid Larvae. Freeze-dried chironomid larvae were obtained from Hikari (CA) and dosed according to the methods described previously.41,42 Briefly, chironomids (120 g per treatment) were added to a preweighed, 3.8 L glass jar and covered with Optima-grade acetone. A precalculated amount of bifenthrin (533 μg/mL in acetone) or fipronil (497 μg/mL in acetone) stock was added for the high-dose treatments, with a 10-fold dilution of stocks and a lower amount used for the low treatment. For the solvent control, an equivalent amount of Optima-grade acetone only was added. All glass jars were capped, briefly mixed, and added to an orbital shaker set at 100 revolutions per min (rpm) (C. MaxQ2000, Thermo Fisher Scientific) for 24 h. Dosed feed was air-dried until a constant mass was achieved.

To assess the concentrations of compounds within dosed food, four replicate subsamples of 0.4 g were taken from each treatment for analysis. The samples were added to a 20 mL scintillation vial, covered with 10 mL of acetone, and 4 ng of DBOFB and DCBP surrogates were added. Further details on the extraction of chironomid larvae and quality assurance/quality control (QA/QC) are available in Sections S1 and S2 of the Supporting Information, respectively. The samples were quantified on an Agilent 7890A gas chromatograph equipped with an Agilent 5975A inert XL MS detector using methane NCI.

2.5. Solid-Phase Extraction of Water Samples. To determine the potential for leaching of contaminants from unconsumed feed and cross-contamination between treatments, SPE was conducted on water samples collected from three randomly selected tanks of each treatment on day 25 using a method adapted from Wang et al.43 and described in detail in Fuller et al.41 Further methodological details for this procedure are available in the Supporting Information (see Section S3). The same method was used to determine the actual concentrations of chlorpyrifos in exposure water for the positive control experiment collected following a 24 and 96 h exposure. Bifenthrin, fipronil, and chlorpyrifos concentrations were quantified using an Agilent 6890A GC equipped with an Agilent 5975A inert XL MS detector using methane NCI.

2.6. Bifenthrin and Fipronil Body Residue Analysis. To assess the actual concentrations of bifenthrin, fipronil, and fipronil biodegradation products in juvenile Chinook salmon, the samples were prepared using accelerated solvent extraction (ASE) followed by freezing lipid precipitation (FLP) and SPE cleanup as described in Fuller et al.41 Fish from each dietary exposure treatment (control, bifenthrin, fipronil, and binary mixture) were included in body residue analysis. Fish were euthanized after a 23-day dietary exposure with any remaining food in the gut removed. Briefly, fish carcasses were freeze-dried for 48–72 h using a Freezone 1 freeze drier (Labconco, Kansas City, MO) and homogenized using a Waring 7010S commercial lab blender (Stanford, CT). ASE was performed by first loading homogenized and freeze-dried juvenile Chinook tissue (2 g) into a stainless-steel cell containing a cellulose filter, copper powder, silica, and lab sand as a filler. The tissue was extracted using an ASE 350 (Thermo Fisher Scientific, Waltham, MA) with two heat-static cycles of an acetone/dichloromethane solution (1:1, v/v) at 100 °C and at 1.03 × 10^7 Pa. The extract was cleaned first with FLP using two 10 mL acetonitrile additions.44 The resulting extract was further cleaned using Envi-carb II/PSA cartridges45 and analyzed by GC-MS.

A set of five or six QA/QC samples were analyzed with each batch of 18 samples: a matrix-free lab blank and a matrix blank sample were used to assess for contamination in the preparation methods, a sample matrix spiked with bifenthrin and fipronil (40 ng) was used to assess the accuracy of the analysis and a matrix spike duplicate sample. A batch duplicate was used to assess the reproducibility of the first batch of fish samples only due to restrictions in the amount of tissue available for extractions. Additional matrix blank or matrix spiked samples were included in various batches; see Section S2 for a summary of QA/QC results for body residue analysis. Sample recovery was assessed by adding DBOFB and PCB-168 surrogates, 40 ng each, to the ASE cell prior to extraction and measuring relative to a spike check.

2.7. Swimming Performance. Maximum swimming speed, \( U_{\text{max}} \), determined using a short-duration constant acceleration test, was selected as a measure of swimming performance in the present study and performed as described by Farrell46 and modified by Fuller et al.41 \( U_{\text{max}} \) was assessed instead of \( U_{\text{crit}} \) due to testing duration while allowing a more robust sample size to be included within a similar size class of juvenile Chinook salmon. Briefly, individual fish were transferred to a 30 L impeller-driven swim tunnel (Loligo, Denmark) containing recirculating dechlorinated municipal water maintained at 11 ± 1 °C. Due to the potential of pesticide carry over from the water or treated fish itself, fish from the same treatment group were tested in succession. This was conducted as a single swim tunnel was available and it was not feasible to wash and cool the chamber after each individual run, only between treatments. Fish were acclimated for 20 min at an approximate speed of 1.5 body lengths (BL) per second and then the water flow was increased every 2 min by 0.2 BL/s until fish were fatigued. Body length measurements were based on measurements of a random subset of fish (n = 20). Fatigue was determined when the fish was impinged against the rear of the swim chamber and did not respond to gentle mechanical stimulation. Following fatigue, the fish was removed from the swim chamber and immediately euthanized with sodium bicarbonate buffered tricaine mesylate (MS-222, 120 mg/L). Swimming performance assays were conducted on a total of 80
fish ($n = 10$ per treatment), including chlorpyrifos-treated fish ($n = 10$). Fish were excluded from analysis if they were unable to swim through the acclimation period or the first velocity increase after acclimation, following the method of Goulding et al.\textsuperscript{46} $U_{\text{max}}$ was calculated according to Farrell (2008).\textsuperscript{45}

### 2.8. Assessment of Liver Damage and Energetics

Following swim performance assays, blood was extracted from Chinook by cardiac puncture, allowed to clot for 30 min at room temperature, centrifuged for 15 min at 2000 x g at 4 °C, and the top serum layer removed, flash frozen in liquid nitrogen, and stored at −80 °C until biochemical assessment. Two biochemical endpoints to assess liver damage and energetics were conducted in treated fish, aspartate aminotransferase (AST) activity, and glucose levels. AST was targeted because AST abundance is the most commonly used biomarker to determine liver damage.\textsuperscript{47,48} Glucose levels were measured because glucose is a critical biomolecule for energetics and shown to be altered by pyrethroids in fish.\textsuperscript{49} AST activity was measured using the Aspartate Aminotransferase Colorimetric Activity Assay Kit (Cayman Chemical, Ann Arbor, MI) and glucose levels were determined using the Glucose Colorimetric Assay Kit (Cayman Chemical) according to the manufacturer’s instructions.

### 2.9. Statistical Analysis

The relationship between swimming performance and biochemical assays relative to bifenthrin and fipronil body residues were tested using linear regression slopes of treatment groups against swimming room temperature, centrifuged for 15 min at 2000 x g. Interaction terms were tested. Di-log normalization skewed data. Two statistical analysis were performed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, N.Y.) to normalize skewed data. Two fish were excluded from the data for failing to swim through the acclimation period. To test for potential synergistic effects of bifenthrin and fipronil mixtures, interaction terms were tested. Differences between the regression slopes of treatment groups against swimming performance and biochemical assays were tested using a t-test as a further measure of potential interactions between bifenthrin and fipronil. Statistically significant differences between groups were inferred if $p < 0.05$.

### 3. RESULTS

#### 3.1. Pesticide Concentrations in Chironomids, Water, and Positive Control

The average measured concentrations for the nominal low (9 ng/g), high (900 ng/g), and bifenthrin and fipronil mixture treatments in the spiked chironomid larvae were as follows: low bifenthrin (10.4 ± 4.18 ng/g, dry weight basis), low fipronil (7.84 ± 1.80 ng/g), high bifenthrin (679 ± 153 ng/g), high fipronil (561 ± 139 ng/g), low mixture (6.69 ± 2.07 ng/g bifenthrin + 4.83 ± 1.87 ng/g fipronil), and high mixture (734 ± 125 ng/g bifenthrin + 575 ± 87.2 ng/g fipronil). The solvent control chironomid treatment group did not have any detectable concentrations of bifenthrin or fipronil, although low amounts of fipronil degradation products were detected across all treatments (Table 1). Low amounts of cypermethrin (range 3.91–4.87 ng/g) and cyhalothrin (range 0.9–1.32 ng/g) were detected in all samples. Background contamination of commercial pellet feed with insecticides has been frequently recorded.\textsuperscript{41,50}

Concentrations of bifenthrin and fipronil parent compounds were either not detected or were below the reporting limit (Table S1) in all water samples collected from aquaria that housed juvenile Chinook salmon throughout the dietary exposure. There were detectable concentrations of the fipronil biotransformation product, fipronil desulfanyl, in the high fipronil and high mixture treatment groups (5.89 ± 4.48 and 10.4 ± 4.18 ng/g bifenthrin + 7.84 ± 1.80 ng/g fipronil). The solvent control chironomid treatment group did not have any detectable concentrations of bifenthrin or fipronil, although low amounts of fipronil degradation products were detected across all treatments (Table 1). Low amounts of cypermethrin (range 3.91–4.87 ng/g) and cyhalothrin (range 0.9–1.32 ng/g) were detected in all samples. Background contamination of commercial pellet feed with insecticides has been frequently recorded.\textsuperscript{41,50}

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#### 3.2. Bifenthrin and Fipronil Body Residues

Average lipid-normalized fish body residues consisted of the following bifenthrin, fipronil (summation of fipronil, fipronil desulfinyl, fipronil sulfide, fipronil sulfoxide), or mixture concentrations in Chinook following 25 days of dietary exposure: control (not detected, nd), low bifenthrin 3.64 ± 0.16 ng bifenthrin/g dry weight, high bifenthrin (343 ± 102 ng bifenthrin/g dry weight), low fipronil (nd), high fipronil (4.11 ± 1.82 ng fipronil/g dry weight), low mixture (2.59 ± 0.73 ng bifenthrin/g dry weight, nd fipronil), and high mixture (155 ± 60.8 ng bifenthrin/g dry weight, 5.05 ± 1.77 ng fipronil/g dry weight) (Table 2).

#### 3.3. Maximum Swimming Speed ($U_{\text{max}}$)

Maximum swimming speed ($U_{\text{max}}$) trials lasted an average of 54 ± 8 min, ranging from 30–85 min across all treatment groups, similar to fatigue times recently reported for juvenile Chinook salmon.\textsuperscript{41} For the aqueous chlorpyrifos positive control, a significant 27%
were nd or BRL in all samples, except one high mix 0.209, F
F

exposure to (A) bifenthrin, (B) fipronil, and mixture treatments (linear regression, were observed for both the bifenthrin and mixture treatments.

Table 2. Average (±Standard Deviation, ng/g Dry Weight) Body Burden Concentrations of Bifenthrin, Fipronil, and Fipronil Sulfone, and Percent Lipid and Dry to Wet Ratios in Juvenile Chinook Salmon Dietarily Exposed to Either a Low (9 ng/g), High (900 ng/g), or a Mixture Treatment for 25 Days

| Treatment            | Lipid (%) | Dry/Wet Ratio | Bifenthrin | Fipronil | Fipronil Sulfone | DBOFB (%) | PCB-168 (%) |
|----------------------|-----------|---------------|------------|----------|------------------|-----------|-------------|
| Solvent Control      | 11.7 ± 2.4| 0.274 ± 0.028 | nd         | nd       | nd               | 108 ± 16.6| 95.9 ± 16.4 |
| Low Bifenthrin       | 9.6 ± 3.1 | 0.297 ± 0.050 | 3.64 ± 1.16| nd       | nd               | 85.4 ± 14.5| 81.4 ± 13.2 |
| High Bifenthrin      | 8.3 ± 2.1 | 0.323 ± 0.044 | 343 ± 102  | nd       | nd               | 86.0 ± 5.9 | 97.7 ± 5.0  |
| Low Fipronil         | 8.5 ± 2.4 | 0.302 ± 0.050 | nd         | nd       | nd               | 82.2 ± 17.3| 81.2 ± 15.0 |
| High Fipronil        | 9.1 ± 2.8 | 0.344 ± 0.036 | nd         | 4.11 ± 1.82| 4.32 ± 1.48     | 98.2 ± 9.7 | 92.9 ± 12.0 |
| Low Mixture          | 10.2 ± 2.5| 0.456 ± 0.094 | 2.59 ± 0.73| nd       | nd               | 98.9 ± 10.0| 97.7 ± 10.3 |
| High Mixture         | 8.9 ± 2.8 | 0.302 ± 0.050 | 155 ± 60.8 | 5.05 ± 1.77| 7.53 ± 2.86     | 88.9 ± 11.4| 90.3 ± 12.1 |

**Figure 1.** Maximum swimming speed ($U_\text{max}$) of juvenile Chinook salmon relative to log-transformed body residues following 25 days of dietary exposure to (A) bifenthrin, (B) fipronil, or (C) mixture of bifenthrin and fipronil, with control exposures represented in each figure. (D) $U_\text{max}$ of juvenile Chinook salmon treated with chlorpyrifos (positive control) for 96 h, relative to solvent control. $U_\text{max}$ is depicted as body lengths per second (BL s$^{-1}$) ($n = 29–30$ for each combined treatment).

3.4. Liver Damage and Energetics Assessment.

Significant positive relationships between log-transformed body residues and AST activity were observed for both the bifenthrin and mixture treatments (linear regression, $r^2 = 0.167$, $F_{1,16} = 5.41$, $p = 0.028$ and $r^2 = 0.209$, $F_{1,28} = 7.40$, $p = 0.011$ for bifenthrin and mixtures, respectively; Figure 1A,C and Table S4), with no significant effect of fipronil residues on $U_\text{max}$ (linear regression, $r^2 = 0.064$, $F_{1,28} = 1.90$, $p = 0.18$; Figure 1B). No significant interaction between bifenthrin and fipronil was observed (linear regression, $p > 0.05$), which was supported by no significant differences between the regression slopes of the bifenthrin, fipronil, and mixture treatments (t-test, $p > 0.05$).
exposed to bifenthrin (linear regression, $r^2 < 0.001$, $F_{1,23} = 0.005$, $p = 0.942$; Figure 2D) or fipronil (linear regression, $r^2 = 0.024$, $F_{1,26} = 0.626$, $p = 0.436$; Figure 2E) alone. No significant interaction between bifenthrin and fipronil was observed, with respect to glucose level (linear regression, $p > 0.05$).

4. DISCUSSION

This is the first known study to assess the effects of dietary exposure to environmentally relevant concentrations of two frequently detected pesticides, bifenthrin and fipronil, and their binary mixture in salmonids. Swimming performance and underlying energy homeostatic pathways were assessed in juvenile Chinook salmon following 25 days of treatment. There was a significant reduction in the maximum swimming speed, $U_{\text{max}}$, in juvenile Chinook salmon dietarily exposed to bifenthrin and a binary mixture of bifenthrin and fipronil. Conversely, exposure to fipronil alone had no significant effect on swimming performance. No significant interaction was observed between bifenthrin and fipronil, suggesting that these two compounds did not act synergistically, and the effects observed in mixture treatments were primarily due to bifenthrin. This was further supported by significantly increased AST activity in bifenthrin and mixture treatments and with increased levels of glucose in the serum of Chinook following exposure to the mixture alone.

Previous studies assessing the effect of aqueous bifenthrin and fipronil treatment on the early life stage of fish demonstrated reduced swimming performance.33 Beggel et al.33 studied the effects of acute (24 h) exposure to bifenthrin and fipronil on the swimming performance of larval fathead minnows using a circular racetrack method.35 Aqueous concentrations of $\geq 0.14 \mu g/L$ of bifenthrin were sufficient to
induce a significant reduction in swimming performance, whereas ≥142 μg/L of fipronil was required to induce a decreased swimming performance.\(^{33}\) Although differences in exposure route, exposure duration, life stage, and species precluded a direct comparison, this supported the findings of the present study, wherein dietary exposure to an average of 0.679 μg/g of bifenthrin alone caused a significant reduction in swimming performance; nevertheless, there was no significant difference in swimming performance after exposure to an average concentration of 0.561 μg/g fipronil alone. When Chinook were exposed to a dietary mixture of bifenthrin and fipronil, there was a similar, significant decrease in swimming performance that was largely driven by bifenthrin, as there was no significant interaction between bifenthrin and fipronil. This suggests that dietary exposure to bifenthrin has a greater toxic response than fipronil on swimming performance. There was also a significantly reduced swimming performance in Chinook treated with the positive control, chlorpyrifos, which has been shown to be a strong inhibitor of swimming performance.\(^{32}\) further validating the use of \(U_{\text{max}}\) as an endpoint for assessing the toxicity of certain pesticide classes. However, due to the low recovery of chlorpyrifos from the exposure water, body burden concentrations might have provided a better representation of concentration that was responsible for the reduction in swimming performance observed, versus the low waterborne measurements detected.

While direct effects on neuromuscular function through receptor or signaling interactions may occur as a result of aqueous exposures to bifenthrin and fipronil, dietary exposures may invoke alternative mechanisms that are involved in energetic pathways. This is likely due to the uptake of pesticides from the gastrointestinal tract and immediate distribution to the liver, which plays a critical role in energetics. Swimming is an energetically taxing process for salmonids with between 2 and 6.7% of the total energy supply required for swimming in Atlantic salmon and a 20% higher energy cost to Chinook salmon.\(^{51}\) Although juvenile Chinook salmon dietarily exposed to 2 μg/g cypermethrin (nominal) for 21 days did not exhibit a significant decrease in swimming speed, there were underlying molecular-level changes in the mRNA expression of two genes, fatty acid synthase (\(fa\)) and ATP citrate lyase (\(acly\)),\(^{41}\) which have important roles in lipid homeostatic function in the liver and energy metabolism. Fathead minnows treated with 350 ng/L bifenthrin had dysregulation of genes involved in neuromuscular and energetic function,\(^{32}\) which was similarly reported in fathead minnows treated with 31–61 μg/L fipronil.\(^{51}\)

The activity level of AST in blood serum is a predominant biomarker of assessing liver injury, which is important in maintaining energy homeostasis.\(^{56,49}\) AST is released as a response to increased liver necrosis following injury or toxic insult. Juvenile Chinook salmon dietarily exposed to bifenthrin alone or as a part of a mixture had significantly increased AST activity. Common carp exposed to a waterborne concentration of 5.75 μg/L bifenthrin had significantly increased levels of AST following a 96 h treatment,\(^{55}\) which was also reported in several additional mammalian and amphibian species following exposure to bifenthrin.\(^{32,53}\) A major role of AST is facilitating glycolysis by oxidizing nicotinamide adenine dinucleotide (NADH), which influences glucose level release in blood following acute injury.\(^{48}\)

An increase in glucose in the blood is a sensitive indicator of stress and an indicator that energy reserves are being intensively utilized,\(^{56}\) with increased glucose levels suggesting a response to deal with increased energy demand in fish following pesticide treatment.\(^{26}\) Glucose levels were significantly increased in juvenile Chinook salmon exposed to the bifenthrin and fipronil mixture. Similarly, Velisek et al.\(^{57}\) found that glucose levels were significantly increased in common carp treated with 5.72 μg/L bifenthrin and also reported increased levels in rainbow trout exposed to 1.47 μg/L bifenthrin for 96 h.\(^{57}\) An increased level of glucose was additionally seen in Nile tilapia (\(Oreochromis niloticus\)) exposed to 0.68 μg/L bifenthrin for 60 days.\(^{58}\) Common carp fry aqueously exposed to 0.142 mg/L fipronil for 15 days also exhibited higher levels of glucose in treated fry, relative to controls.\(^{59}\) The consistent increase in plasma glucose in multiple fish species following bifenthrin treatment suggests that bifenthrin may alter hepatic lipid and energy homeostatic processes that may impair proper swimming performance in salmonids. Although increased glucose levels represent a final product from a dysregulated homeostatic balance of energetic processes, assessing additional biochemical endpoints and gene expression profiles to further characterize alternative effects occurring within the liver, such as those previously measured in juvenile Chinook salmon dietarily exposed to cypermethrin, would be helpful to fully understand what may be driving changes to energy metabolism.

In contrast, fipronil exposure alone did not alter glucose levels in treated Chinook salmon, nor did fipronil alter levels when dosed with bifenthrin as a mixture. Differences in the bioaccumulation and elimination of bifenthrin and fipronil may be a factor in the large differences in toxicity and effects observed between the two compounds in the present study. Juvenile Chinook salmon exposed to 679 ng/g bifenthrin for 25 days accumulated average lipid-normalized body residues of 343 ± 102 ng bifenthrin/g dry weight, whereas fish exposed to 561 ng/g fipronil for the same duration accumulated average residues of 4.11 ± 1.82 ng fipronil/g dry weight (reported as the sum of parent fipronil and biodegradation products fipronil sulfone, fipronil desulfanyl, and fipronil sulfide, which are known to be more toxic to fish than the parent compound).\(^{60}\) Additional toxicokinetic studies in other salmonids exposed to fipronil recorded rapid biotransformation and elimination of fipronil. Konwick et al.\(^{30}\) exposed rainbow trout to 7.68 μg/L fipronil for a period of 32 days, followed by a 96-day depuration period. Fipronil had the shortest biological half-life of 0.61 days compared with 10 legacy contaminants (organochlorines and PCBs), as well as the lowest absorption efficiency of 23 ± 2%.\(^{30}\)

Although comparative studies for dietary bifenthrin accumulation and elimination in fish are scarce, a study of bluegill, \(Lepomis macrochirus\), exposed to aqueous \(^{14}\)C-bifenthrin for 60 days observed a biological half-life of 22 days.\(^{61}\) Bifenthrin has been shown to bioaccumulate in prey items consumed by juvenile Chinook salmon,\(^{9,36}\) emphasizing the potential for dietary exposure at similar concentrations to the present study during rearing. Huff Hartz et al.\(^{1}\) recorded bifenthrin concentrations of up to 813 ng/g lipid bifenthrin (equivalent to 13.6 ng/g on a dry mass basis) in pyrethroid-resistant populations of the epibenthic amphipod, \(Hyalella azteca\), a known component of juvenile Chinook salmon diets,\(^{57}\) collected from northern California. Measured bifenthrin residues in the low treatment in the present study were comparable at 10.4 ng/g, which in turn led to measurable residues of 3.64 ± 1.16 ng/g dry weight after 25 days of...
exposure in juvenile Chinook salmon, highlighting the potential for bifenthrin bioaccumulation in the natural environment. Changes in salinity within the Delta likely influences the bioavailability and uptake potential of bifenthrin and fipronil into the diets of juvenile Chinook and should be considered in future studies. Bioaccumulation of bifenthrin has been confirmed in wild-caught fish; Corcelles et al. documented bioaccumulation of bifenthrin from 0.64 to 81.4 ng/g lipid in several fish species collected from Iberian rivers.

Thus, differences in toxicokinetic parameters, such as biotransformation and elimination, may have contributed to the observed differences in effects in the present study, with bifenthrin being eliminated much more slowly as compared to fipronil, leading to higher residues in the liver, potentially altering energetics and subsequently impacting swimming performance. Additionally, the bioaccumulation of bifenthrin was nearly twofold lower in the binary mixture treatment relative to bifenthrin alone, which could reflect a change in the metabolic process when co-exposed to fipronil, with additional research warranted to assess the potential interaction between bifenthrin and fipronil and relation to metabolism. Furthermore, data from acute aqueous studies suggest that bifenthrin may be more toxic to salmonids than fipronil. Acute 96 h LC50 values for bifenthrin and fipronil in rainbow trout, Oncorhynchus mykiss, have been found to be 0.15 and 246 μg/L, respectively, suggesting that bifenthrin is more toxic to salmonids by over 3 orders of magnitude. The finding that bifenthrin bioaccumulation has a significant negative effect on swimming performance and biochemical processes involved in energetic function, coupled with the known field bioaccumulation of pyrethroids in fish, has implications for foraging activity and predator avoidance in juvenile salmonids in the wild.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c06609.

Extraction of dosed chironomid larvae (S1 Text); bifenthrin and fipronil body residue analysis and QA/QC (S2 Text); solid-phase extraction of water samples (S3 Text); Concentrations of bifenthrin, fipronil, and fipronil biotransformation products in water (Table S1); concentration of chlorpyrifos in water (Table S2); QA/QC parameters for chlorpyrifos exposures (Table S3); and Umax values, AST activity, and glucose levels used for generating regression analysis relative to log normalized body residues (Tables S4–S6) (PDF)

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