EPA Public Access

Author manuscript

Comp Biochem Physiol C Toxicol Pharmacol. Author manuscript; available in PMC 2019 February 03.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Comp Biochem Physiol C Toxicol Pharmacol. 2018 February ; 205: 34–42. doi:10.1016/j.cbpc.

Toxicokinetics of the neonicotinoid insecticide imidacloprid in rainbow trout (*Oncorhynchus mykiss*)

John A. Frew^{a,*}, Jacob T. Brown^b, Patrick N. Fitzsimmons^c, Alex D. Hoffman^c, Martin Sadilek^d, Christian E. Grue^a, and John W. Nichols^c

^aSchool of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, United States

^bCollege of Pharmacy, University of Minnesota, Duluth, MN, United States

^cMid-Continent Ecology Division, U.S. Environmental Protection Agency, Duluth, MN, United States

^dDepartment of Chemistry, University of Washington, Seattle, WA, United States

Abstract

Imidacloprid (IMI) is the largest selling insecticide internationally. Little is known about the toxicokinetics of IMI in fish, however. *In vivo* time-course studies were conducted to study the distribution and elimination of IMI in rainbow trout. Animals confined to respirometer-metabolism chambers were injected with a low (47.6 µg/kg), medium (117.5 µg/kg) or high (232.7 µg/kg) dose directly into the bloodstream and allowed to depurate. The fish were then sampled to characterize the loss of IMI from plasma and its appearance in expired water (all dose groups) and urine (medium dose group only). *In vitro* biotransformation of IMI was evaluated using trout liver S9 fractions. The plasma time-course data indicated an early (< 12 h) distributional phase followed by a log-linear terminal elimination phase. Mean total clearance (CL_T) values determined by non-compartmental analysis were 21.8, 27.0 and 19.5 mL/h/kg for the low, medium and high dose groups, respectively. Estimated half-lives for the same groups were 67.0, 68.4 and 68.1 h, while mean fitted values for the steady-state volume of distribution (V_{SS}) were 1.72, 2.23 and 1.81 L/kg. Measured branchial elimination rates were much lower than expected, suggesting that IMI is highly bound in blood. Renal clearance rates were greater than measured rates of branchial clearance (60% of CL_T in the medium dose group), possibly indicating a role for renal membrane transporters. There was no evidence for hepatic biotransformation of IMI. Collectively, these findings suggest that IMI would accumulate in trout in continuous waterborne exposures.

Keywords

Imidacloprid; Neonicotinoids; Rainbow trout; Fish; Toxicokinetics; Renal clearance; Biotransformation; Bioaccumulation

*Corresponding author: frew.john@gmail.com.

1. Introduction

Neonicotinoids are systemic insecticides used for crop protection against plant-sucking insects, and for the control of fleas and ticks on pets (Jeschke et al., 2011; Kanne et al., 2005; Tomizawa and Casida, 2003). These compounds act as agonists at the acetylcholine binding sites on nicotinic acetylcholine receptors (nAChRs) and display highly selective toxicity owing to structural differences between invertebrate and vertebrate nAChRs (Jeschke et al., 2011; Tomizawa and Casida, 2003; Tomizawa et al., 2003). Beginning in the early 1990s, increased pest resistance, combined with increasingly stringent use restrictions, resulted in reduced use of organophosphate and methylcarbamate insecticides. Neonicotinoids have largely replaced these compounds, and are now the most widely used insecticides (Gibbons et al., 2015; Jeschke et al., 2011; Tomizawa and Casida, 2003).

Imidacloprid (IMI; N-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl] nitramide), the first neonicotinoid insecticide brought to market, is the largest selling insecticide internationally (Jeschke et al., 2011). The extensive use of IMI has resulted in widespread contamination of surface waters (Gibbons et al., 2015; van der Sluijs et al., 2015). Although aqueous photolytic degradation under laboratory conditions is relatively rapid (half-life = 1.2–2.1 h; (Moza et al., 1998; Wamhoff and Schneider, 1999)), IMI may persist in environmental settings. The freshwater half-lives of IMI in rice field studies in sunlight and darkness were 4 d and 10–24 weeks, respectively (Sanchez-Bayo and Goka, 2005).

The presence of IMI in surface waters has raised concerns about its toxicity to fish (Gibbons et al., 2015). Acute median lethal concentration (LC₅₀) values for fish range from 1.2 mg/L for rainbow trout (*Oncorhynchus mykiss*) fry (Cox, 2001) to 240 mg/L for zebrafish (*Danio rerio*) (Tisler et al., 2009). Environmental concentrations typically range from 2–7 orders of magnitude lower than these LC₅₀ values (Gibbons et al., 2015). With the exception of extreme circumstances, therefore, exposure to IMI is unlikely to result in acute lethality to fish (Gibbons et al., 2015).

Sublethal effects of IMI on fish have been reported at environmental concentrations and durations of exposure (Gibbons et al., 2015). Histological changes were observed in the testes of Nile tilapia (*Oreochromis niloticus*), potentially resulting in developmental and reproductive effects, following IMI exposure at a concentration of 0.136 mg/L (Lauan and Ocampo, 2013). Immunotoxic effects were detected in experimental rice fields. Stress from exposure to IMI at 0.03–0.24 mg/L resulted in ectoparasite infestations in juvenile Medaka (*Oreochromis niloticus*) (Sanchez-Bayo and Goka, 2005).

IMI is a neutral compound possessing a relatively low log K_{OW} value of 0.57 (Fossen, 2006). These properties suggest that IMI has only a moderate degree of hydrophobic character. The molecular weight of IMI is 255.7. Based on these characteristics, one may reasonably predict that IMI would diffuse easily across the gill epithelium and into fish tissues, but exhibit little tendency to partition non-specifically to tissue lipids and proteins. However, there are currently no published studies on the toxicokinetics of IMI in fish. It is difficult, therefore, to relate observed effects in fish following a defined exposure to an

absorbed dose, ideally expressed as the chemical concentration time-course in target tissues. In the present study, large rainbow trout confined to respirometer-metabolism chambers were administered a bolus intra-arterial dose of IMI and allowed to depurate. The objectives of the study were to determine the apparent volume of distribution and total clearance of IMI, investigate the major routes of clearance and their significance to whole-body elimination, and characterize the distribution of IMI in select tissues.

2. Material and methods

2.1. Chemicals

IMI (99.5% pure) was purchased from Chem Service Inc. (West Chester, PA). Deuterium-labeled IMI internal standard (> 98% pure) was purchased from Sigma- Aldrich (St. Louis, MO). High performance liquid chromatography (HPLC) grade acetonitrile, methanol and sodium chloride were purchased from Fisher Scientific (Pittsburgh, PA). Tricaine methanesulfonate (MS-222) was purchased from Argent Laboratories (Redmond, WA). β -nicotinamide adenine dinucleotide phosphate (β - NADPH; > 95% pure) was purchased from the Oriental Yeast Co. (Tokyo, Japan). Adenosine 3'-phosphate 5'-phosphosulfate (PAPS; 80% pure) was obtained from EMD Millipore (Billerica, MA). All other chemicals and solvents were purchased from Sigma-Aldrich and were reagent grade or higher in quality.

2.2. Animals

Rainbow trout (*Oncorhynchus mykiss*) were obtained from the US Geological Survey Upper Midwest Environmental Science Center (La Crosse, WI) and raised to the necessary size for these studies. Fish were maintained in sand-filtered Lake Superior water on a natural photoperiod and fed commercial trout chow (Classic Trout; Skretting USA, Tooele, UT). Water quality characteristics were measured and recorded daily. Temperature was held at 11 ± 1 °C, dissolved oxygen at 85–100% saturation, pH at 7.6–7.8, total ammonia at < 1 mg/L, water hardness (as CaCO₃) at 45–46 mg/L, and alkalinity at 41–44 mg/L. All experiments were performed in accordance with guidelines set by the USEPA Mid-Continent Ecology Division Animal Care and Use Committee.

2.3. Surgical preparation

Fish were surgically prepared for containment in individual respirometer-metabolism chambers, as described previously (McKim and Goeden, 1982). Each animal was anesthetized with MS-222 and cannulated from the dorsal aorta to permit serial blood sampling. A latex oral membrane was sewn over the fish's mouth to separate inspired and expired water flows. A second membrane, located just posterior to the gills, separated the expired water compartment from a third, whole body compartment. Urinary catheters were implanted in fish from the medium dose group for continuous collection of urine. Ventilation volume (V_{VOL} ; mL/min/kg) throughout each study using an automated data collection system (Carlson, 1989).

2.4. Bolus dosing studies

The plasma time-course of IMI was evaluated in 3 groups of chambered trout ("low", "medium", and "high" dose) following administration of a bolus intra-arterial dose. The IMI

dosing solution was prepared using Cortland's physiological saline (Wolf, 1963). Nominal doses for the low ($n = 5$), medium ($n = 8$), and high ($n = 5$) groups were 45, 100, and 250 $\mu\text{g/kg}$ body weight (bw), respectively, and were administered in 1.0 mL/kg bw of dosing solution.

Blood samples (50 or 100 μL) were collected before dosing to assess background IMI concentrations. Additional samples were then collected at 1, 2, 4, 8, 16, 24, 36 and 48 h post-injection (low and high dose groups), or at 0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24 and 36 h post-injection (medium dose group). Blood samples were withdrawn from the dorsal aortic cannula using heparinized capillary tubes and immediately transferred to 200 μL picocentrifuge tubes. Following termination of the experiment, 3–5 mL of blood was withdrawn from the caudal vein and split into 3 or more 1.5 mL microcentrifuge tubes, depending on the volume collected. Blood was centrifuged for 10 min at $5000 \times g$ (4°C) to obtain plasma. The plasma was transferred to clean 200 μL picocentrifuge tubes or 1.5 mL microcentrifuge tubes (terminal samples), flash-frozen in liquid N_2 and stored at -80°C . Samples were then shipped to the University of Washington (UW) on dry ice and stored at -80°C until IMI residue analysis.

Approximately 200 mL of water was collected in 250 mL Nalgene bottles from the outflow tube of the expired water chamber concurrent with blood sampling at each time interval. Collected water was stored in the dark at 4°C for a maximum of 48 h prior to residue concentration utilizing reversed-phase solid phase extraction (SPE) as described below. The concentrated IMI water samples were dried in 15 mL conical tubes and packed in dry ice for shipment to the UW. The samples were then stored at -80°C until IMI residue analysis.

Urine was collected in 15 or 50 mL conical tubes during the interval between blood sampling times (medium dose group only). The volume of urine produced during this time period was recorded. Subsamples were transferred to 1.5 mL microcentrifuge tubes (maximum of 10 per time interval) and stored at -20°C following collection. At completion of the experiments, all samples were shipped overnight on dry ice to the UW and stored at -80°C until IMI residue analysis.

Animals were anesthetized in MS-222 at 36 or 48 h post-injection and euthanized by exsanguination. Each fish was then processed to obtain the brain, kidney, and liver, and a representative sample of white muscle. Bile, which tends to be retained in the gallbladder of chambered fish, also was collected. These samples were flash-frozen in liquid N_2 and stored at -80°C . All samples were shipped to the UW on dry ice and stored at -80°C until IMI residue quantitation.

2.5. Hepatic metabolism study

A pooled sample lot containing liver S9 fractions from 5 trout of mixed sex was prepared following an established protocol (Johanning et al., 2012). This material was then aliquoted (0.5 mL) into 1.8 mL cryogenic storage tubes and frozen at -80°C prior to use. To confirm activity, thawed S9 samples were characterized to determine 7-ethoxyresorufin-*O*-deethylase activity (EROD; a surrogate for CYP1A activity), uridine diphosphate glucuronosyltransferase (UGT) activity, and glutathione-*S*-transferase (GST) activity.

Methods used to perform these assays are described elsewhere (Nichols et al., 2013). The protein content of the pooled S9 sample was determined using Peterson's modification of the Lowry method (Sigma technical bulletin TP0300; Sigma Aldrich). All characterization assays and protein determinations were performed in duplicate. Heat-inactivated material, used as a negative control, was obtained by boiling S9 fractions for 10 min (Johanning et al., 2012).

In vitro biotransformation of IMI was evaluated at 11 ± 1 °C using a substrate depletion approach (Nichols et al., 2013). Incubations were performed in 200 µL reaction mixtures containing 100 mM potassium phosphate buffer (pH 7.80 ± 0.05), 1 mg/mL S9 protein, 2 mM uridine 5'-diphosphoglucuronic acid (UDPGA), 2 mM β-NADPH, 5 mM reduced L-glutathione (GSH), and 0.1 mM PAPS. Alamethicin (10 µg/mL), a pore-forming peptide, was added to support UGT activity (Ladd et al., 2016). Reactions were initiated by adding IMI in potassium phosphate buffer (10 µL) and terminated with the addition of 600 µL of ice-cold acetonitrile. The tested (nominal) concentration of IMI was 1 µM. Duplicate samples containing active protein were terminated at 0, 20, 40, 60, 80, 100 and 120 min. Additional samples containing denatured S9 protein were incorporated to account for possible non-metabolic losses such as volatilization or photo-degradation. The terminated samples were vigorously mixed and centrifuged for 5 min at $3000 \times g$ (4 °C) to pellet protein. Supernatants were transferred to 2-mL amber GC vials and stored at 4 °C for 48 h prior to being shipped to the UW on dry ice. The samples were then stored at -80 °C until IMI residue quantitation.

2.6. Analytical methods

2.6.1. Internal standard solution—A 1000 µg/L internal standard solution prepared in DI water was integrated into samples prior to residue quantitation. All samples were spiked with 25 µL of the internal standard solution. The nominal concentration of internal standard following reconstitution 50µg/L.

2.6.2. Plasma and bile samples—Methods for processing plasma and bile samples were adapted from an established protocol (Ford and Casida, 2006). Plasma (0.025–0.065 g) and bile (0.222–0.226 g) samples were transferred to 50 mL conical tubes and spiked with internal standard. The samples were then allowed to equilibrate in the dark at 4 °C for 2 h. Following equilibration, 5 mL acetonitrile and 250 mg NaCl were added. The samples were placed on ice and homogenized using a sonic dismembrator (Model 4C15, Fisher Scientific, Pittsburgh, PA) set at maximum power for 2 min, followed by vigorous vortexing for 1 min. The supernatant was collected after centrifugation for 15 min at $4000 \times g$ (22 °C), transferred to a glass evaporation tube, and dried under N₂ in a vacuum manifold with drying attachment (Supelco Visiprep and Visidry, Sigma-Aldrich). Dried samples were reconstituted in 0.5 g 30:70 acetonitrile/DI water prior to quantitation.

2.6.3. Tissue samples—Tissues were removed from -80 °C storage the day of processing and allowed to thaw to room temperature. Thawed tissues (1 per fish for each tissue type) were placed on disposable plastic weight trays and minced with a clean #20 scalpel. The samples were transferred to 50 mL conical tubes and masses were recorded.

Roughly equivalent masses of DI water were added for approximately 2-fold sample dilutions. The samples were homogenized with a rotor/stator type tissue homogenizer (Tissue Tearor Model 398, Biospec Products, Bartlesville, OK) set at maximum power for 2–3 min. Subsamples of homogenate (~0.25 g) were transferred to 50.0 mL conical tubes and spiked with internal standard. Internal standard equilibration, tissue disintegration via sonic dismembration, and extraction/drying of the supernatant were performed as described for plasma and bile samples. Dried samples reconstituted in 0.5 g 30:70 acetonitrile/DI water prior to quantitation.

2.6.4. Expired water samples—The low concentrations of IMI present in expired water required concentration of the samples. Water samples (one subsample, 100 mL, per fish for each sampling time) were decanted into reservoirs fitted onto 4.0 mL, 200 mg bed weight SPE columns (Grace C₁₈ Extract Clean, Grace Division Discovery Sciences, Columbia, MD). The decanted water samples were spiked with internal standard. Reversed phase SPE and methanol eluent drying under N₂ were performed using the aforementioned vacuum manifold with drying attachment following the manufacturer's recommended protocol. Dried samples were reconstituted in 0.5 g 30:70 acetonitrile/DI water prior to quantitation.

2.6.5. Urine samples—Urine samples (one subsample, 0.5 mL, per fish for each sampling time) were transferred to 4.0 mL, 200 mg bed weight SPE columns and spiked with internal standard. Reversed phase SPE and methanol eluent drying were performed as described above. Dried samples were reconstituted in 0.5 g 30:70 acetonitrile/DI water prior to quantitation.

2.6.6. S9 fractions—Active and denatured S9 fraction/acetonitrile solutions were transferred to 2.0 mL cylindrical tubes. Containers were rinsed with 0.5 mL acetonitrile and transferred to the cylindrical tubes. The S9/acetonitrile solutions were spiked with internal standard and centrifuged for 10 min at 5000 × *g* (22 °C). Supernatant was transferred in its entirety via pipet to evaporation tubes and dried under N₂ using a vacuum manifold with drying attachment. Dried samples were reconstituted in 0.5 g 30:70 acetonitrile/DI water prior to quantitation.

2.6.7. Quantitation—IMI residues were quantitated using LC-MS detection through adaptation of an existing protocol (Schoning and Schmuck, 2003). Samples were analyzed on a 2795 Alliance HT-quaternary system HPLC/Quattro Micro triple quadrupole mass spectrometer (Waters Corp., Milford, MA). A Zorbax SB-C18 narrow bore RR 2.1 × 100 mm 3.5 micron HPLC column (Agilent Technologies, Inc., Santa Clara, CA) was used for analyte separation. Injection volume was 10 µL and oven temperature was 30 °C. Mobile phase A was DI water, 5% acetonitrile, 1% acetic acid. Mobile phase B was 99% acetonitrile, 1% acetic acid. Mobile phase C was isopropanol. The interface was electrospray, capillary voltage +3.5 kV, with desolvation temperature of 300 °C. Scan type was multiple reaction monitoring mode. Polarity was positive. The collision gas was argon. A partial loop injection of 10 µL of sample was combined with wash solution (50% IPA, 50% methanol – MeOH) and purge solution (10% ACN, 90% DI water) in the 50 µL HPLC sample loop. The 12-min gradient timetable was as follows: an initial mobile phase of 68%

solution A, 32% solution B at a flow rate of 200 $\mu\text{L}/\text{min}$ from time interval 0–4.5 min; 52% solution A, 48% solution B, at a flow rate of 200 $\mu\text{L}/\text{min}$ from time interval 4.5–6.5 min; 1% solution A, 65% solution B, and 34% solution C at a flow rate of 350 $\mu\text{L}/\text{min}$ from 6.5–9.0 min; 32% solution B and 68% solution C at a flow rate of 350 $\mu\text{L}/\text{min}$ from 9.0–11.0 min, with the flow rate reduced to 200 $\mu\text{L}/\text{min}$ from 11.0–12.0 min. The analyte retention time window was approximately 2.6–3.0 min. Analytical sensitivity of blank samples had a limit of quantification (LOQ) of 1.0 $\mu\text{g}/\text{L}$ and limit of detection (LOD) of 0.3 $\mu\text{g}/\text{L}$.

2.7. Modeling and statistical analysis

Pharmacokinetic analyses were performed using Kinetica version 5.1 (Thermo Scientific, Philadelphia, PA). Non-compartmental pharmacokinetic parameters were estimated for each subject based on individual plasma concentration vs. time data (Gabrielsson and Weiner, 2012). Measured plasma concentrations were logtransformed and plotted against time to determine the terminal elimination phase for each animal, typically covering 4–6 sampling time points. The half-life ($t_{1/2}$; h) for IMI was determined for each subject as $\text{Ln}(2)$ divided by the fitted terminal elimination rate constant (λ_z ; 1/h). The area under the plasma concentration vs. time curve during the sampling period (AUC_{0-t} ; $\mu\text{g}/\text{L}/\text{h}$) was calculated using the mixed log-linear rule and extrapolated to infinity ($\text{AUC}_{0-\infty}$; $\mu\text{g}/\text{L}/\text{h}$). Total clearance (CL_T ; $\text{L}/\text{h}/\text{kg}$) was calculated as the weight-based dose divided by $\text{AUC}_{0-\infty}$. The apparent volume of distribution during the terminal phase (V_Z ; L/kg) was calculated as the dose divided by the product of $\text{AUC}_{0-\infty}$ and λ_z , while the apparent steady-state volume of distribution (V_{SS} ; L/kg) was calculated as the product of CL_T and mean residence time (MRT; h). The AUC for IMI in expired water was calculated using GraphPad Prism 6 (GraphPad Software, La Jolla, CA).

Measured concentrations of IMI from the *in vitro* substrate depletion study were log-transformed and plotted against time. The data were then analyzed by simple linear regression, and the slopes determined for both active samples and denatured negative controls. The statistical package for Excel (Microsoft Corporation, Redmond, WA) was used for all statistical calculations.

3. Results

3.1. Ventilation volume

Measured V_{VOL} values were averaged across all sampling times to calculate a mean for each animal. The means for each fish in a dosing group were then averaged to calculate an overall group mean. Respective mean values ($\pm$ SD) for the low, medium, and high dose groups were 11.2 ± 2.9 , 14.0 ± 3.1 , and 13.3 ± 2.5 $\text{L}/\text{h}/\text{kg}$. Similar values have been reported in previous studies with chambered trout (Consoer et al., 2014, 2016; Fitzsimmons et al., 2009).

3.2 Background concentrations

The background concentrations of IMI in plasma, urine, and expired water samples from each animal collected before dosing were below the LOQ. Similarly, background

concentrations of IMI in bile, brain, kidney, liver, and muscle, collected from an unexposed animal were below the LOQ.

3.3. Bolus dose IMI elimination/excretion

The actual delivered doses, determined by measuring IMI concentrations in each dosing solution, were 47.6, 117.5, and 232.7 $\mu\text{g/kg}$ bw for the low, medium, and high dose groups, respectively. The mass of IMI injected into each animal (μg) is given in Table 1.

Measured IMI concentrations in plasma for all study animals suggested the existence of an early distributional phase (< 12 h), followed by a log-linear terminal elimination phase (Figure 1). Modeled kinetic parameters describing the disposition of IMI in plasma are presented in Table 1. The mean $t_{1/2}$ for each dosing group was generally similar to or somewhat longer than the total study period (ranging from 41.7 h to 63.8 h). In addition, $\text{AUC}_{0-\infty}$ values for individual animals tended to be larger than the AUC_{0-t} , with the result that the $\text{AUC}_{\text{extra}}$ ($\text{AUC}_{0-\infty} - \text{AUC}_{0-t}$) was typically between 30% and 70% of $\text{AUC}_{0-\infty}$. Under these circumstances, small errors in estimation of λ_z may translate to large errors in estimation of $\text{AUC}_{0-\infty}$. As a result, confidence in calculated values of CL_T and V_{SS} is reduced (Gabrielsson and Weiner, 2012).

Nevertheless, kinetic parameters for all tested animals exhibited remarkable consistency. One-way ANOVA comparisons ($\alpha = 0.05$) therefore conducted under the null hypothesis that mean CL_T , $t_{1/2}$, and V_{SS} values were the same in all 3 dosing groups. The null hypothesis was confirmed for CL_T and $t_{1/2}$ ($p = 0.38$ and 0.99 , respectively), but not for V_{SS} ($p = 0.0039$). Fitted V_{SS} values did not exhibit any obvious dose-dependence, however. Averaged across all 18 animals, the mean V_{SS} (1.97 L/kg) was approximately 2.8 times greater than the average total body water (TBW) volume of 714 mL/kg for freshwater teleosts (Thorson, 1961).

Measured IMI concentrations in expired water were used to create concentration-time profiles for each dosing group (Figure 2). A similar concentration-time profile was developed using urine data from the medium dose group (Figure 3). Expired water profiles developed for the medium and high dose groups provided weak evidence for biphasic elimination of IMI, while that developed for the low dose group was impossible to decipher. Challenges associated with making these measurements may have obscured true patterns of elimination, however. In contrast, the urine profile mirrored the biphasic elimination pattern suggested by the plasma concentration time-course data.

The mass of IMI excreted across the gills during the course of an experiment was calculated for the medium and high dose groups (a similar calculation for the low dose group was not possible due to incongruities in the data). The AUC was calculated for each animal based on measured concentrations of IMI in expired water at each depuration time interval. The total mass of IMI was then calculated by multiplying the AUC for each animal by its mean V_{VOL} . Branchial excretion (mean $\pm$ SD) for the medium (36 h depuration) and high (48 h depuration) dose groups averaged 18.7 ± 3.7 and 50.8 ± 7 μg ; accounting for $17.2 \pm 3.9\%$ and $23.9 \pm 4.6\%$ of the administered dose, respectively.

The mass of IMI excreted in urine during each time interval was calculated as the product of the measured IMI concentration and the known volume of the sample. These masses were then summed across all time intervals to determine the total mass excreted by each fish. Cumulative renal excretion (mean $\pm$ SD; medium dose group only) across the 36 h depuration period averaged 28.9 ± 10.0 μ g, accounting for $27.1 \pm 11.1\%$ of the administered dose.

3.4. Tissue distribution

Measured concentrations of IMI (mean $\pm$ SD) in plasma, brain, kidney, liver, muscle, and bile from exposed animals are shown in Table 2. The table also presents the measured concentrations of IMI in urine during the last collection interval (24 to 36 h) for the medium dose group. Tissue:plasma concentration ratios for the muscle and brain were similar, while those for liver and kidney were somewhat higher. Concentration ratios determined for bile and urine were generally higher than those for liver and kidney, though not markedly so.

3.5. Hepatic metabolism study

The pooled sample of liver S9 fractions had a total protein content of 26.3 ± 0.4 mg/mL. Measured levels of EROD and UGT activity for this sample were 11.3 ± 1.7 pmol/min mg protein and 1075 ± 198 pmol/min mg protein, respectively. The measured level of GST activity was 444 ± 47 nmol/min mg protein. All of these values are comparable to values determined previously for S9 sample lots obtained from the same strain of trout (Fay et al., 2017; Nichols et al., 2013). There was no apparent loss of IMI from active or denatured samples during the 2 h incubation period (slopes indistinguishable from 0, $\alpha = 0.05$). As such, the *in vitro* S9 assay provided no evidence for hepatic metabolism of IMI by trout.

3.6. Mass balance

A chemical mass-balance was calculated for each medium dose fish, from which both expired water and urine data were obtained ($n = 4$; Table 3). The mass of IMI in the body at the end of depuration was estimated as the product of the modeled volume of distribution at the end of the 36 h depuration period (V_Z) and the plasma concentration at 36 h. Masses excreted across the gills and in urine were calculated as described in Section 3.3 (*Bolus dose IMI elimination/excretion*). Averaged across all 4 animals, the chemical mass balance (mean $\pm$ SD) was $97 \pm 21\%$ of the administered dose. These results indicate that branchial and renal excretion are the predominant routes of elimination, with the former contributing approximately 40% and the latter roughly 60% to total excretion.

4. Discussion

The goal of this investigation was to characterize the distribution and elimination of IMI in rainbow trout. Study results indicated that IMI injected into the bloodstream distributes rapidly throughout the body. Because it is a relatively polar compound, IMI might have been expected to distribute primarily to body water. This would have resulted in a V_{SS} approximately equivalent to TBW (0.714 mL/kg; (Thorson, 1961)). Instead, the mean V_{SS} for each dosing group was approximately 250–300% greater than TBW. This modeled result indicates that IMI has higher affinity for tissues than it does for blood plasma, and suggests

some degree of extra- or intra-cellular tissue binding (Bertelsen et al., 1998; Rowland and Tozer, 2005).

The V_{SS} may be interpreted as the sorption capacity of the fish relative to that of plasma (Nichols et al., 2006). White muscle constitutes approximately 50% of rainbow trout body weight (Goolish, 1989; Webb and Johnsrude, 1988). Measured muscle:plasma concentration ratios may therefore be utilized to evaluate the overall chemical sorption capacity of the fish, provided that a chemical distributes extensively to this tissue and does not accumulate to high levels elsewhere. Measured IMI residues in muscle and plasma were consistent with this suggestion: the range of measured muscle:plasma concentration ratios for the three study groups (1.6–2.2) were almost identical to the fitted V_{SS} values (range of group means from 1.72 to 2.23 L/kg).

IMI was eliminated almost entirely by excretion of parent compound across the gills or into urine. Absent significant diffusion limitations, and assuming no contribution by active transporters in the gills, the rate of branchial elimination is limited by the capacity of blood to deliver chemical to the gills or the capacity of respired water to carry this chemical away from the gills, whichever is smaller (Erickson and McKim, 1990). For a compound that is completely unbound in blood, the capacity of blood to deliver chemical to the gills is approximately equal to the cardiac output (Q_C), which in large trout is approximately 2 L/h/kg (Nichols et al., 1990). In theory, this is the maximum rate of whole-body clearance that can be achieved by this route of elimination. When a chemical is highly bound in blood, branchial elimination is limited instead by the capacity of water to carry chemical away from the gills. Under these conditions, the clearance rate may be approximated as the rate of water flow across the gills (about 60% of V_{VOL} ; (Nichols et al., 1990)), divided by a chemical's equilibrium blood:water partition coefficient (P_{BW}). For highly bound chemicals, these considerations may result in branchial elimination rates that are a small fraction of those for unbound chemicals (Erickson and McKim, 1990).

Previously, (Fitzsimmons et al., 2001) developed a log K_{ow} -based algorithm that predicts P_{BW} for neutral chemicals in trout, based on their tendency to partition non-specifically to lipids and non-lipid organic matter:

$$P_{BW} = (10^{0.73 \log K_{ow}} \times 0.16) + 0.84 \quad (1)$$

Assuming further that chemical dissolved in the aqueous portion of blood is unbound, P_{BW} values may be related to the unbound chemical fraction in blood (f_U) by the relationship (Nichols et al., 2006):

$$f_U = v_{WBL} / P_{BW}; \quad (2)$$

where v_{WBL} is the fractional water content of blood (assumed here to be 0.84; (Bertelsen et al., 1998)). Applying these relationships, the calculated P_{BW} for IMI is 1.26, while calculated f_U is 0.67. Thus, based on its relative hydrophobicity, IMI is predicted to be

largely unbound in trout blood. From this, it may be predicted that whole-body clearance due to branchial elimination would be a substantial fraction of Q_C . However, an examination of Table 1 suggests instead that fitted CL_T values were 100 times lower than Q_C (range of group means from 0.0195–0.0270 L/h/kg. This finding indicates that IMI is highly bound in trout blood, which suggests in turn that it binds specifically to molecular components of blood.

A search of the literature failed to identify any measured f_U values for IMI in fish or mammals. However, several authors have studied IMI binding to human serum albumin and hemoglobin (Ding et al., 2010; Ding and Peng, 2015; Wang et al., 2009). These studies indicate that IMI exhibits moderate affinity for both molecules, resulting in binding constants (K_A) that range from $1\text{--}2 \times 10^4$ L/mol. In either case, this binding appears to be associated with a single molecular binding site. Trout possess multiple plasma proteins that bear a resemblance to human albumin (Maillou and Nimmo, 1993). The affinity of IMI for these proteins is unknown; nevertheless, the fact that IMI exhibits substantial affinity for both hemoglobin and albumin in humans provides a plausible explanation for low branchial clearance rates in trout.

If the 60% renal contribution to total modeled clearance is applied to all 3 dosing groups, calculated renal clearance rates (CL_R) range from 11.7 to 16.2 mL/h/kg. These CL_R values are approximately 2 to 3 times higher than measured glomerular filtration rates in large trout (5.4 to 6.1 mL/h/kg; Consoer et al., 2014; McKim et al., 1999). This finding, combined with the likelihood that IMI is highly bound in plasma, suggests that renal membrane transporters actively secrete IMI in the kidney. To our knowledge, studies of IMI transport in the mammalian kidney are lacking. However, membrane transporters were shown to be responsible for polarized transport (basal to apical) in the human intestinal Caco-2 cell line (Brunet et al., 2004). This transport was ATP-dependent, but appeared to be distinct from classical ABC-transport systems such as P-glycoproteins (P-gp) and multidrug resistance associated proteins (MRP).

Bile:plasma concentration ratios determined in trout were relatively high (range 5.1 to 7.9), suggesting that IMI is secreted into the bile. However, studies with rats indicate that the oral bioavailability of IMI is high (96%; (Klein, 1987)). Assuming a comparable degree of oral bioavailability in trout, IMI secreted into bile and released from the gallbladder would probably be reabsorbed. Therefore, excretion in bile is unlikely to be a significant route of elimination.

The results of the present study provide insight into similarities and differences in the kinetics of IMI in fish and mammals. The rapid distribution of IMI in trout following i.a. injection is consistent with observations from dosing experiments in mammals. Peak concentrations of IMI in mouse brain and liver were recorded at the first sampling interval (15 min) following intraperitoneal (i.p.) injection (Ford and Casida, 2006). Extensive distribution of radiolabeled IMI to tissues and organs was measured in rats 5 min after intravenous (i.v.) injection, and 1 h after oral dosing (Klein, 1987).

When radiolabeled IMI is administered to mammals, most of the radioactivity appears in urine. However, only a small fraction is present as the parent compound (22% and 12% of the administered doses in mice and rats, respectively; (Ford and Casida, 2006; Karl and Klein, 1992; Klein and Karl, 1990)). Instead, most of this radioactivity is present as metabolites. In contrast to trout, therefore, biotransformation is the primary route of IMI elimination in mammals. Multiple cytochrome P450 isozymes from human liver are selective for oxidation or reduction of IMI (Schulz-Jander and Casida, 2002). Aldehyde oxidase has been identified as the enzyme responsible for the reduction of IMI in rabbit liver cytosol (Dick et al., 2005).

To our knowledge, this is the first study of IMI kinetics in fish. The results suggest some similarities in handling of IMI by trout and mammals (e.g., rapid distribution) as well as striking differences (e.g., an apparent absence of biotransformation in trout). Importantly, branchial clearance of IMI by trout was much lower than would have been predicted based on non-specific partitioning to blood constituents. This was likely due to specific binding of IMI to plasma proteins and/or hemoglobin. This latter finding is significant insofar as it suggests that IMI would tend to accumulate in trout in continuous aqueous exposures.

The extent of chemical accumulation in fish resulting from a water-only exposure is commonly expressed in terms of a bioconcentration factor (BCF), which is defined as the ratio of chemical concentrations in the fish and water under steady-state conditions. For many chemicals, the BCF may be estimated kinetically using a one-compartment model, where k_1 (L/h/kg) is the branchial uptake rate constant, and k_2 (1/h) is a whole-body elimination rate constant representing all processes responsible for chemical elimination. At steady-state, the rate of uptake equals the rate of elimination and the $BCF = k_1/k_2$ (No, 2012). Given the apparent binding of IMI in blood, the branchial uptake rate may be expected to approach the flow rate of water through gill lamellar channels (referred to as the respiratory volume, Q_W ; (Erickson and McKim, 1990)). In large trout, Q_W is approximately 60% of V_{VOL} (Erickson and McKim, 1990). For trout evaluated in the present study, this equates to 12.8 L/h/kg (mean, all groups) times 0.6, or 7.7 L/h/kg.

It is clear that the kinetics of IMI elimination following bolus i.a. injection are multi-exponential. However, the distributional phase (<12 h) is a small fraction of the time that would be required for fish to achieve steady-state in a continuous waterborne exposure (given a $t > 60$ h). In a long-term exposure, therefore, complexity associated with chemical distribution within the animal can be largely ignored, and the ratio of CL_T and V_{SS} approximates the elimination rate constant k_2 from the one-compartment model (Rowland and Tozer, 2005). The respective average CL_T and V_{SS} values for all 3 dosing groups were roughly 0.02 L/h/kg, and 2 L/kg, resulting in an estimated $k_2 = 0.01$ /h. Assuming, as discussed above, that IMI is taken up efficiently across the gills, the BCF predicted for large trout is therefore 7.7 L/h/kg divided by 0.01/h, or 770 L/kg.

In order to place this kinetically-determined BCF into context, the calculated value may be compared to a BCF predicted from non-specific partitioning of IMI to lipids and proteins. Using an established algorithm (Arnot and Gobas, 2004), this partitioning-based BCF (BCF_p) may be estimated from a compound's K_{OW} value as:

$$BCF_P = \gamma_{LB} K_{OW} + \gamma_{NB} \beta K_{OW} + \gamma_{WB} \quad (3)$$

where γ_{LB} (unitless) is the fractional lipid content of the organism, γ_{NB} (unitless) is the fractional content of non-lipid organic material, γ_{WB} (unitless) is the fractional water content, and β (unitless) is a proportionality constant that reflects the sorption capacity of non-lipid organic material relative to that of lipid. For this assessment, the TBW value of 0.714 (Thorson, 1961) was adopted as an estimate of γ_{WB} , and γ_{LB} was set equal to the fractional lipid content determined previously for large trout (0.098; (Nichols et al., 1990)). The value of γ_{NB} was then calculated as $1 - \gamma_{LB} - \gamma_{WB}$, or 0.188, and β was set equal to the value 0.05 (Debruyne and Gobas, 2007). Based on these assignments, the calculated BCF_P is approximately 1.1. Thus, the BCF predicted from measured rates of elimination and an estimated rate of branchial uptake is approximately 700 times higher than that predicted from simple partitioning considerations.

The results of this study suggest that IMI does not behave in trout in the manner expected for a compound that diffuses passively across biological membranes and partitions non-specifically to blood and tissues. Instead, IMI appears to possess attributes, including specific binding in plasma and renal clearance by active secretion, exhibited by a number of ionized compounds (Armitage et al., 2017). Given the widespread use of IMI, as well as its documented presence and relative persistence in surface waters, additional research on uptake and accumulation in fish appears to be warranted.

Acknowledgments

This document has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

- Armitage JM, Erickson RJ, Luckenbach T, Ng CA, Prosser RS, Arnot JA, Schirmer K, Nichols JW. Assessing the bioaccumulation potential of ionizable organic compounds: current knowledge and research priorities. *Environmental toxicology and chemistry*. 2017; 36:882–897. [PubMed: 27992066]
- Arnot JA, Gobas FA. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environmental Toxicology and Chemistry*. 2004; 23:2343–2355. [PubMed: 15511097]
- Bertelsen SL, Hoffman AD, Gallinat CA, Elonen CM, Nichols JW. Evaluation of log K_{ow} and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environmental Toxicology and Chemistry*. 1998; 17:1447–1455.
- Brunet JL, Maresca M, Fantini J, Belzunces LP. Human intestinal absorption of imidacloprid with Caco-2 cells as enterocyte model. *Toxicol Appl Pharmacol*. 2004; 194:1–9. [PubMed: 14728974]
- Carlson, RWL., GW, Holmen, BA. An automated monitoring system for fish physiology and toxicology. U.S. Environmental Protection Agency; Duluth, MN: 1989. EPA/600/3-89/011
- Consoer DM, Hoffman AD, Fitzsimmons PN, Kosian PA, Nichols JW. Toxicokinetics of perfluorooctanoate (PFOA) in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol*. 2014; 156:65–73. [PubMed: 25150511]

- Consoer DM, Hoffman AD, Fitzsimmons PN, Kosian PA, Nichols JW. Toxicokinetics of perfluorooctane sulfonate in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem*. 2016; 35:717–727. [PubMed: 26332333]
- Cox C. Insecticide factsheet: imidacloprid. *J Pestic Reform*. 2001; 21:15–21.
- Debruyne AM, Gobas FA. The sorptive capacity of animal protein. *Environmental Toxicology and Chemistry*. 2007; 26:1803–1808. [PubMed: 17705657]
- Dick RA, Kanne DB, Casida JE. Identification of aldehyde oxidase as the neonicotinoid nitroreductase. *Chem Res Toxicol*. 2005; 18:317–323. [PubMed: 15720138]
- Ding F, Han BY, Liu W, Zhang L, Sun Y. Interaction of imidacloprid with hemoglobin by fluorescence and circular dichroism. *J Fluoresc*. 2010; 20:753–762. [PubMed: 20204684]
- Ding F, Peng W. Biological assessment of neonicotinoids imidacloprid and its major metabolites for potentially human health using globular proteins as a model. *J Photochem Photobiol B*. 2015; 147:24–36. [PubMed: 25837412]
- Erickson RJ, McKim JM. A model for exchange of organic chemicals at fish gills: flow and diffusion limitations. *Aquatic Toxicology*. 1990; 18:175–197.
- Fay KA, Fitzsimmons PN, Hoffman AD, Nichols JW. Comparison of trout hepatocytes and liver S9 fractions as in vitro models for predicting hepatic clearance in fish. *Environ Toxicol Chem*. 2017; 36:463–471. [PubMed: 27487575]
- Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC, Nichols JW. Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol*. 2001; 55:23–34. [PubMed: 11551619]
- Fitzsimmons PN, Hoffman AD, Lien GJ, Hammermeister DE, Nichols JW. Kinetics and effects of dichloroacetic acid in rainbow trout. *Aquat Toxicol*. 2009; 94:186–194. [PubMed: 19656582]
- Ford KA, Casida JE. Chloropyridinyl neonicotinoid insecticides: diverse molecular substituents contribute to facile metabolism in mice. *Chem Res Toxicol*. 2006; 19:944–951. [PubMed: 16841963]
- Fossen, M. Environmental fate of imidacloprid. California Department of Pesticide Regulation; 2006. p. 1-16.
- Gabrielsson J, Weiner D. Non-compartmental analysis. *Computational Toxicology: Volume I*. 2012:377–389.
- Gibbons D, Morrissey C, Mineau P. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res Int*. 2015; 22:103–118. [PubMed: 24938819]
- Goolish EM. The scaling of aerobic and anaerobic muscle power in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology*. 1989; 147:493–505.
- Jeschke P, Nauen R, Schindler M, Elbert A. Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem*. 2011; 59:2897–2908. [PubMed: 20565065]
- Johanning K, Hancock G, Escher B, Adekola A, Bernhard MJ, Cowan-Ellsberry C, Domoradzki J, Dyer S, Eickhoff C, Embry M, Erhardt S, Fitzsimmons P, Halder M, Hill J, Holden D, Johnson R, Rutishauser S, Segner H, Schultz I, Nichols J. Assessment of metabolic stability using the rainbow trout (*Oncorhynchus mykiss*) liver S9 fraction. *Curr Protoc Toxicol*. 2012; Chapter 14(Unit 14 10): 11–28.
- Kanne DB, Dick RA, Tomizawa M, Casida JE. Neonicotinoid nitroguanidine insecticide metabolites: synthesis and nicotinic receptor potency of guanidines, aminoguanidines, and their derivatives. *Chem Res Toxicol*. 2005; 18:1479–1484. [PubMed: 16167841]
- Karl W, Klein O. Pyridinyl-14C-methyl] imidacloprid: Distribution of the metabolites in some organs at different times following single oral administration to rats. 1992 Unpublished report from Bayer AG, report No. PF 3635.
- Klein, O. INCHEM Toxicological Evaluations: Imidacloprid. Mannheim, Germany: 1987. [14C]-NTN 33893: Biokinetic part of the 'General metabolism study' in the rat.
- Klein, O., Karl, W. Leverkusen-Bayerwerk, Germany, Study. 1990. Methylene-[14 C] Imidacloprid: Metabolism Part of the General Metabolism Study in the Rat. Bayer AG; p. 51950-50021.

- Ladd MA, Fitzsimmons PN, Nichols JW. Optimization of a UDP-glucuronosyltransferase assay for trout liver S9 fractions: activity enhancement by alamethicin, a pore-forming peptide. *Xenobiotica*. 2016; 46:1066–1075. [PubMed: 26947351]
- Lauan M, Ocampo P. Low-dose effects of carbaryl, chlorpyrifos and imidacloprid on the gonad and plasma testosterone level of male juvenile and adult Nile tilapia (*Oreochromis niloticus* Linnaeus). *Asia Life Sci*. 2013; 1:239–250.
- Maillou J, Nimmo I. Identification and some properties of an albumin-like protein in the serum of pre-spawning Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. 1993; 104:401–405.
- McKim JM, Goeden HM. A direct measure of the uptake efficiency of a xenobiotic chemical across the gills of brook trout (*Salvelinus fontinalis*) under normoxic and hypoxic conditions. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*. 1982; 72:65–74.
- McKim JM, Kolanczyk RC, Lien GJ, Hoffman AD. Dynamics of renal excretion of phenol and major metabolites in the rainbow trout (*Oncorhynchus mykiss*). *Aquatic toxicology*. 1999; 45:265–277.
- Moza P, Hustert K, Feicht E, Kettrup A. Photolysis of imidacloprid in aqueous solution. *Chemosphere*. 1998; 36:497–502.
- Nichols JW, Hoffman AD, ter Laak TL, Fitzsimmons PN. Hepatic clearance of 6 polycyclic aromatic hydrocarbons by isolated perfused trout livers: prediction from in vitro clearance by liver S9 fractions. *Toxicol Sci*. 2013; 136:359–372. [PubMed: 24097670]
- Nichols JW, McKim JM, Andersen ME, Gargas ML, Clewell HJ 3rd, Erickson RJ. A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. *Toxicol Appl Pharmacol*. 1990; 106:433–447. [PubMed: 2260091]
- Nichols JW, Schultz IR, Fitzsimmons PN. In vitro–in vivo extrapolation of quantitative hepatic biotransformation data for fish: I. A review of methods, and strategies for incorporating intrinsic clearance estimates into chemical kinetic models. *Aquatic toxicology*. 2006; 78:74–90. [PubMed: 16513189]
- No, T. OECD Guidelines for the Testing of Chemicals, Section 3. 2012. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure.
- Rowland, M., Tozer, TN. Clinical pharmacokinetics/pharmacodynamics. Lippincott Williams and Wilkins; Philadelphia: 2005.
- Sanchez-Bayo F, Goka K. Unexpected effects of zinc pyrethrin and imidacloprid on Japanese medaka fish (*Oryzias latipes*). *Aquat Toxicol*. 2005; 74:285–293. [PubMed: 16023744]
- Schoning R, Schmuck R. Analytical determination of imidacloprid and relevant metabolite residues by LC MS/MS. *Bulletin of Insectology*. 2003; 56:41–50.
- Schulz-Jander DA, Casida JE. Imidacloprid insecticide metabolism: human cytochrome P450 isozymes differ in selectivity for imidazolidine oxidation versus nitroimine reduction. *Toxicol Lett*. 2002; 132:65–70. [PubMed: 12084621]
- Thorson TB. The partitioning of body water in Osteichthyes: phylogenetic and ecological implications in aquatic vertebrates. *The Biological Bulletin*. 1961; 120:238–254.
- Tisler T, Jemec A, Mozetic B, Trebse P. Hazard identification of imidacloprid to aquatic environment. *Chemosphere*. 2009; 76:907–914. [PubMed: 19505710]
- Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annu Rev Entomol*. 2003; 48:339–364. [PubMed: 12208819]
- Tomizawa M, Zhang N, Durkin KA, Olmstead MM, Casida JE. The neonicotinoid electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the *Drosophila* nicotinic receptor: an anomaly for the nicotinoid cation– π interaction model. *Biochemistry*. 2003; 42:7819–7827. [PubMed: 12820891]
- van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bijleveld van Lexmond MF, Bonmatin JM, Chagnon M, Downs CA, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EA, Morrissey CA, Noome DA, Pisa L, Settele J, Simon-Delso N, Stark JD, Tapparo A, Van Dyck H, van Praagh J, Whitehorn PR, Wiemers M. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environ Sci Pollut Res Int*. 2015; 22:148–154. [PubMed: 25296936]

- Wamhoff H, Schneider V. Photodegradation of imidacloprid. *J Agric Food Chem.* 1999; 47:1730–1734. [PubMed: 10564045]
- Wang YQ, Tang BP, Zhang HM, Zhou QH, Zhang GC. Studies on the interaction between imidacloprid and human serum albumin: spectroscopic approach. *J Photochem Photobiol B.* 2009; 94:183–190. [PubMed: 19126446]
- Webb PW, Johnsrude C. The effect of size on the mechanical properties of the myotomal-skeletal system of rainbow trout (*Salmo gairdneri*). *Fish physiology and biochemistry.* 1988; 5:163–171. [PubMed: 24226676]
- Wolf K. Physiological salines for fresh-water teleosts. *The Progressive Fish- Culturist.* 1963; 25:135–140.

Fig. 1.

Log-transformed plasma elimination profiles for rainbow trout following bolus intra-arterial injections of low (47.6 µg/kg bw), medium (117.5 µg/kg bw), and high (232.7 µg/kg bw) doses of imidacloprid (IMI). Plasma was sampled at selected times over 36 h (medium dose group) and 48 h (low and high dose groups). Squares, circles, and triangles denote low ($n = 5$; except at 16 h, where $n = 2$), medium ($n = 8$; except at 6 and 12 h, where $n = 7$), and high ($n = 5$) dose groups, respectively. Values are reported as the mean $\pm$ SD.

Fig. 2.

Measured expired water concentrations from trout following intra-arterial injections of low (47.6 µg/kg bw), medium (117.5 µg/kg bw), and high (232.7 µg/kg bw) doses of imidacloprid (IMI). Expired water was sampled at selected times over 36 h (medium dose group) and 48 h (low and high dose groups). Squares, circles, and triangles denote low ($n = 5$; except at 16 h, where $n = 3$; and 48 h, where $n = 4$), medium ($n = 8$; except 1, 6, and 36 h, where $n = 7$), and high ($n = 5$; except at 1 h, where $n = 4$; 16 h, where $n = 2$; 36 h, where $n = 3$; and 48 h, where $n = 4$) dose groups, respectively. Values are reported as the mean $\pm$ SD.

Fig. 3. Measured urine concentrations from trout following intra-arterial injections of a medium (117.5 µg/kg bw) dose of imidacloprid (IMI). Values are reported as the mean $\pm$ SD; $n = 4$; except at 0.5 and 24 h, where $n = 3$.

Table 1

Kinetic parameters from depuration studies with chambered trout.

	Low dose					Mean $\pm$ SD
	Fish L1	Fish L2	Fish L3	Fish L4	Fish L5	
Sex	male	female	female	female	female	
Weight (kg)	0.73	0.70	0.89	0.83	0.78	0.79 $\pm$ 0.08
Delivered dose (μ g)	34.7	33.3	42.4	39.5	37.1	37.4 $\pm$ 3.6
Kinetic parameters						
AUC ₀₋₄ (μ g/L/h)	1124.6	884.1	1337.8	1075.9	982.1	1080.9 $\pm$ 170.6
AUC _{0-∞} (μ g/L/h)	2324.5	1498.3	3479.1	2883.1	1812.8	1318.6 $\pm$ 644.9
AUC _{extra} (%)	51.6	41.0	61.5	62.7	45.8	52.5 $\pm$ 9.5
V _{ss} (L/kg)	1.70	1.77	1.64	1.79	1.68	1.72 $\pm$ 0.07
V _{ss} (%TBW)	238	248	230	251	235	240 $\pm$ 9
V _Z (L/kg)	2.02	2.06	1.87	1.87	1.83	1.93 $\pm$ 0.10
CL(L/h/kg)	0.0205	0.0318	0.0137	0.0165	0.0263	0.0218
t _{1/2} (h)	68.5	45.1	94.6	78.5	48.3	67.0 $\pm$ 20.8
	Mednm dose					Mean $\pm$ SD
	Fish M1	Fish M2	Fish M3	Fish M4	Fish M5	
Sex	male	female	male	female	female	
Weight (kg)	0.96	0.81	0.88	1.09	0.87	0.96 $\pm$ 0.09
Delivered dose (μ g)	113.2	95.2	103.4	128.1	101.8	109.2 $\pm$ 11.2
Kinetic parameters						
AUC ₀₋₄ (μ g/L/h)	1250.7	1302.4	1379.9	1899.5	1802.0	1561.8 $\pm$ 381.4
AUC _{0-∞} (μ g/L/h)	3000.7	2967.5	2839.5	6829.3	8236.9	5405.2 $\pm$ 2608.7
AUC _{extra} (%)	58.3	56.1	51.4	72.2	78.1	67.3 $\pm$ 10.4
V _{ss} (L/kg)	2.64	2.49	2.28	1.92	2.15	2.23 $\pm$ 0.3
V _{ss} (% TBW)	370	349	319	269	301	312 $\pm$ 42
V _Z (L/kg)	2.67	2.53	2.33	1.93	2.17	2.25 $\pm$ 0.31
CL(L/h/kg)	0.0392	0.0396	0.0414	0.0172	0.0143	0.0270 $\pm$ 0.0127

	Low dose					High dose					Mean ± SD
	Fish L1	Fish L2	Fish L3	Fish L4	Fish L5	Fish H1	Fish H2	Fish H3	Fish H4	Fish H5	
$t_{1/2}$ (h)	47.3	44.3	39.0	77.9	105.6	77.1	51.2	104.4	68.4 ± 26.8		
Sex	Fish H1 female	Fish H2 female	Fish H3 female	Fish H4 female	Fish H5 male						Mean ± SD
Weight (kg)	0.84	1.05	0.85	0.95	0.93						0.92 ± 0.09
Delivered dose (µg)	196.2	244.3	198.3	220.1	216.2						215.0 ± 19.5
Kinetic parameters											
AUC_{0-1} (µg/L/h)	5110.3	5813.1	4813.2	4526.3	4470.8						4946.8 ± 547.4
$AUC_{0-\infty}$ (µg/L/h)	14031.7	14003.6	9152.8	10476.4	13934.6						12319.8 ± 2334.6
AUC_{extra} (%)	63.6	58.5	47.4	56.8	67.9						58.8 ± 7.7
V_{ss} (L/kg)	1.79	1.52	1.64	1.94	2.16						1.81 ± 0.25
V_{ss} (% TBW)	251.00	213.00	230.00	272.00	302.00						254 ± 35
V_z (L/kg)	1.81	1.54	1.68	2.01	2.21						1.85 ± 0.26
CL (L/h/kg)	0.0166	0.0166	0.0254	0.0222	0.0167						0.0195
CL_b (L/h/kg)	0.0126	0.0066	0.0121	0.0142	0.0118						0.0115
$t_{1/2}$ (h)	75.8	64.4	45.9	62.8	91.6						68.1 ± 17.0

Table 2

Distribution of IMI to selected tissues and organs of chambered rainbow trout at the end of depuration following bolus intra-arterial injection. Data presented as mean $\pm$ SD.

Sample	Low dose (48 h depuration, n = 5)		Medium dose (36 h depuration, n = 8)		High dose (48 h depuration, n = 5)	
	IMI concentration	Tissue:plasma ratio	IMI concentration	Tissue:plasma ratio	IMI concentration	Tissue:plasma ratio
Plasma	13.3 $\pm$ 2.9		33.4 $\pm$ 13.0		76.4 $\pm$ 8.1	
Brain	19.6 $\pm$ 2.6	1.4 $\pm$ 0.1	54.0 $\pm$ 14.0	1.8 $\pm$ 0.4	98.5 $\pm$ 16.9	1.3 $\pm$ 0.1
Kidney	44.7 $\pm$ 6.0	3.2 $\pm$ 0.3	292.9 $\pm$ 102.7	9.8 $\pm$ 4.6	257.9 $\pm$ 32.3	3.4 $\pm$ 0.4
Liver	41.9 $\pm$ 7.9	3.0 $\pm$ 0.4	1143 $\pm$ 21.4	3.9 $\pm$ 1.5	228.9 $\pm$ 32.3	3.0 $\pm$ 0.3
Muscle	22.4 $\pm$ 1.8	1.7 $\pm$ 0.3	67.0 $\pm$ 18.1	2.2 $\pm$ 0.7	118.8 $\pm$ 12.4	1.6 $\pm$ 0.1
Bile	69.3 $\pm$ 5.2	5.1 $\pm$ 1.0	230.8 $\pm$ 57.4	7.9 $\pm$ 3.1	442.2 $\pm$ 75.5	5.6 $\pm$ 0.5
Urine	NA	NA	1185 $\pm$ 40.4	5.0 $\pm$ 1.9	NA	NA

Table 3

Chemical mass-balance for each medium dose fish from which both expired water and urine data were obtained.

Fish	Delivered dose (µg)	Renal excretion (µg)	Branchial excretion (µg)	Mass remaining in body (µg)	Mass balance (%)
M1	113.2	22.9	24.5	72.0	105.6
M3 [*]	103.4	21.2	14.9	39.9	73.4
M7	98.9	43.1	16.6	25.0	85.6
M5	118.8	28.5	17.4	98.5	121.5
Mean ± SD	108.6 ± 9.0	28.9 ± 10.0	18.3 ± 4.3	58.8 ± 32.9	96.5 ± 21.3

* 0–24 h branchial excretion only