Comparative Biochemistry and Physiology, Part C 205 (2018) 34–42

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

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

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ARTICLE INFO

Keywords:
Imidacloprid
Neonicotinoids
Rainbow trout
Fish
Toxicokinetics
Renal clearance
Biotransformation
Bioaccumulation

ABSTRACT

Studies were conducted to determine the distribution and elimination of imidacloprid (IMI) in rainbow trout. Animals 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 only). In vitro biotransformation of IMI was evaluated using trout liver S9 fractions. Mean total clearance (CL_T) values determined by non-compartmental analysis of plasma time-course data 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 fitted values for the steady-state volume of distribution (V_{SS}) were 1.72, 2.23 and 1.81 L/kg. 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.

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-chloropyridyn-3-yl)methyl]-4,5-dyhydroimidazol-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 (Morrissey et al., 2015; Sánchez-Bayo et al., 2016). 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 wks, respectively (Sanchez-Bayo and Goka, 2005; Wamhoff and Schneider, 1999).

The presence of IMI in surface waters has raised concerns about its toxicity to fish (Gibbons et al., 2015). Acute median lethal concentration (LC_{50}) values for fish range from 1.2 mg/L for rainbow trout (*Oncorhynchus mykiss*) fry (Cox, 2001) to 241 mg/L for zebrafish (*Danio rerio*) (Tisler et al., 2009), although most LC_{50} values listed in the U.S. Environmental Protection Agency (USEPA) ECOTOX database are between 100 and 200 mg/L. Environmental concentrations typically range from 2 to 7 orders of magnitude lower than these LC_{50} 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).

However, sublethal effects of IMI on fish have been reported at

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https://doi.org/10.1016/j.cbpc.2018.01.002
Received 18 October 2017; Received in revised form 17 January 2018; Accepted 21 January 2018

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 (*Oryzias latipes*) (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. Limited studies with fish support this suggestion. For example, Ding et al. (2004) exposed zebrafish to IMI in water for 14 days (Ding et al., 2004). Whole-body bioconcentration factors (BCFs) calculated by these authors ranged from 0.97 to 1.52. Iturburu et al. (2017) calculated tissue-specific BCFs for IMI in chameleon cichlids (*Australoheros facetus*) following short-term (24 or 48 h) waterborne exposures (Iturburu et al., 2017). Most of these BCFs ranged between 0.2 and 2.0.

The foregoing studies provide little information, however, on the kinetics of IMI in fish, and no data regarding processes that control uptake and elimination. 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, adult rainbow trout confined to respirometer-metabolism chambers were administered a bolus intra-arterial (i.a.) 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 (D4)-labeled IMI internal standard (> 98% pure) was purchased from Sigma-Aldrich (St. Louis, MO). High performance liquid chromatography (HPLC) grade acetonitrile (ACN), methanol (MeOH), isopropanol (IPA) 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 (approximately 0.7 to 1.0 kg) 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 $CaCO_3$) 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. All fish were fasted throughout each experiment beginning 24 h before surgery.

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) was monitored throughout each study using an automated data collection system (Carlson et al., 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 i.a. 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 μg/kg, respectively, and were administered in 1.0 mL/kg of dosing solution. These dosing levels were designed to achieve IMI concentrations in blood, urine, and expired water that were above analytical limits of detection, while covering a modest range of concentrations in order to evaluate the concentration-dependence of elimination. For comparison, a kinetic study of IMI in rats was performed by administering an intravenous dose of 1 mg/kg (Klein, 1987).

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 × 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 on 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