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Bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

There are very little data on the bioaccumulation and biotransformation of current-use pesticides (CUPs) despite the fact that such data are critical in assessing their fate and potential toxic effects in aquatic organisms. To help address this issue, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to dietary concentrations of a mixture of chiral triazole fungicides (bromuconazole, cyproconazole, metconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole, and triadimefon) and a chiral legacy pesticide [α-hexachlorocyclohexane (α-HCH)] to study the bioaccumulation and biotransformation of these CUPs. Fish accumulated all triazoles rapidly during the 8 day uptake phase, and was followed by rapid elimination, which was estimated by taking accelerated sampling times during the 16 day depuration period. Half-lives ($t_{1/2}$s) and times to 95% elimination (t_{95}s) ranged from 1.0 to 2.5 and 4.5 to 11.0 days, respectively. Chiral analysis suggested no significant selectivity in biotransformation for most of the compounds based on statistically unaltered enantiomer fractions (EFs) in the fish compared to food values; exceptions were a change in EF of myclobutanil and changes in diastereomer fractions (DFs) of propiconazole and cyproconazole. No biotransformation was observed for α-HCH based on consistent EFs in the fish throughout the experiment and a $t_{1/2}$ (15.8 days) that fell within the 95% confidence interval of a log K_{ow}–log $t_{1/2}$ relationship developed for assessing biotransformation of organic contaminants. This relationship did show that biotransformation accounted for the majority (ranging from 59.9 to 90.4%) of the elimination for all triazoles, and that triazole compounds with oxygen and hydroxyl functional groups were more easily biotransformed. This research indicated that chiral analysis may potentially miss biotransformation of CUPs and other potential non-persistent organic contaminants and shows the utility of the log K_{ow}–log $t_{1/2}$ relationship as a mechanistic tool for quantifying biotransformation. Based on the rapid biotransformation of the triazoles, future research should focus on formation of metabolites and their fate and possible effects in the environment.

Keywords: Enantiomers; Diastereomers; Current-use pesticides; Conazoles

1. Introduction

Current-use pesticides (CUPs) (e.g., atrazine, fipronil, diazinon, permethrin, propiconazole) can be defined as those modern pesticides that are currently registered for use, generally developed from chemical synthesis, and are typically used in the agricultural or lawn care sector. They are generally less environmentally persistent having shorter half-lives and lower bioaccumulation than the organochlorine pesticides that they have replaced. There is concern over the wide application of CUPs and their possible detrimental effects on aquatic ecosystem health that may arise from spray drift or surface run-off after rainfall events. Of particular importance are readily formed *in vivo* metabolites of CUPs, which may cause greater harm in aquatic biota than their parent compound (Sinclair and Boxall, 2003). Thus, for accurate risk assessment, there is a need to characterize the persistence and accumulation of CUPs in aquatic biota, including biotransformation rates to metabolites. However, there have been few studies that have addressed this issue for CUPs (Konwick et al., 2006), likely due to their short environmental persistence in biota (i.e., low log K_{ow} values) and the inherent difficulty in identifying the numerous potential biotransformation products. In addition, models based on physical–chemical properties of contaminants to estimate biotransformation rates in fish are limited (Borgå et al., 2004), especially concerning CUPs.

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In 1996, it was estimated that approximately 25% of CUPs were chiral (Williams, 1996), with that number to likely have risen since then because of the increasing complexity of modern pesticides with the greater likelihood of chiral centers. Chiral chemicals exist as two non-superimposable mirror images called enantiomers, often designated as (+) and (−) based on their rotation of plane-polarized light. The manufacture of chiral chemicals results in a racemic (±) mixture containing an equal percentage (50%) of each enantiomer, the form in which they are typically released into the environment. Chemicals can also contain more than one chiral center and thus exist in other stereoisomeric forms. For example, a chemical with two chiral centers would exist as two diastereomers; these are not mirror images of each other, but each would consist of two enantiomers. Enantiomers, unlike diastereomers, have identical physical–chemical (i.e., achiral) properties (Garrison, 2006), and only show differences in selectivity when in a chiral environment. Thus, the relative abundance of enantiomers is subject to change after metabolic processes due to numerous enzymes and receptors having symmetry (i.e., chiral) dependence; this property has been used recently as a tracer for biotransformation (Vetter et al., 2001; Wong et al., 2004). For example, non-racemic enantiomer residues (expressed as enantiomer ratios or fractions) have indicated that fish can biotransform several chiral organochlorines (OCs) as well as CUPs (Wong et al., 2002; Konwick et al., 2006). The use of changes in relative proportions of diastereomers has rarely been used as a biotransformation tracer, but has similar potential to enantiomer ratios. However, this feature may be compromised by the fact that diastereomers can undergo abiotic reactions at different rates.

Another method based on a curve-linear relationship developed between $\log K_{ow}$ and $t_{1/2}$ for a series of recalcitrant PCBs in juvenile rainbow trout (Fisk et al., 1998) can also provide a means of assessing biotransformation, specifically rates, in fish (Fisk et al., 2000; Buckman et al., 2006; Konwick et al., 2006). Based on this method, non-recalcitrant chemicals whose $t_{1/2}$ (determined experimentally using the same protocol) fall below this curve-linear relationship are suggested to be biotransformed, whereas those chemicals that fall on or near this relationship are assumed to not undergo biotransformation. This method has been used to generate biotransformation rates for several polychlorinated alkanes and PCBs, and fipronil in juvenile rainbow trout (Fisk et al., 2000; Buckman et al., 2006; Konwick et al., 2006), with the potential application to other CUPs.

Triazoles as well as the structurally related imidazole fungicides are used as clinical drugs and as agricultural pesticides, including applications for the treatment and protection of cereals, soybeans, and a variety of fruits (Roberts and Hutson, 1999). Their fungicidal effect is a result of the inhibition of cytochrome (CYP) P-450 dependent C14 demethylation of lanosterol, an intermediate in ergosterol biosynthesis (Roberts and Hutson, 1999). Other studies have shown that the inhibition of CYP forms is not limited to sterol biosynthesis (Rodrigues et al., 1988; Ronis et al., 1998). In fish, CYP mediated steroid metabolism (Monod et al., 1993), in addition to xenobiotic metabolism (Levine et al., 1999a; Hegelund et al., 2004), can be altered. In fish exposed to propiconazole, for example, a mixed pattern response in metabolism occurs, whereby CYP1A mRNA levels increase, but EROD activity decreases (Levine et al., 1999a). This alteration in metabolism can lead to potentially higher bioaccumulation and toxicity of contaminants. For example, gizzard shad (*Dorosoma cepedianum*) pre-exposed to clotrimazole had greater bioaccumulation of benzo[*a*]pyrene compared to fish exposed to benzo[*a*]pyrene only (Levine et al., 1997). In addition, fathead minnows (*Pimphales promelas*) that were pre-treated with propiconazole showed enhanced acute toxicity after exposure to the pesticide parathion (Levine and Oris, 1999b). Therefore, it is important to understand the fate and toxicokinetics of these fungicides in fish, in part because of their ability to increase the accumulation and toxicity to other contaminants.

We investigated the bioaccumulation and biotransformation of a series of triazole fungicides by dietary exposure to juvenile rainbow trout (*Oncorhynchus mykiss*) to address the lack of such information for CUPs. The ability of this fish to biotransform these chemicals was assessed through chiral analysis and the use of the $\log K_{ow}$–$\log t_{1/2}$ relationship developed for quantifying biotransformation of organic contaminants in rainbow trout (Fisk et al., 1998, 2000). α-HCH was included in this study to expand the $\log K_{ow}$–$\log t_{1/2}$ relationship to lower $\log K_{ow}$ chemicals. In addition, we attempted to explore what role the functional groups attached to the chiral center of the triazoles had on biotransformation. To our knowledge, this is the first experiment to investigate the bioaccumulation and enantioselective biotransformation of triazole fungicides in fish.

2. Materials and methods

2.1. Chemicals and food preparation

All triazoles were obtained from the EPA Repository (EPA National Pesticide Standard Repository, Ft. Meade, MD). α-HCH was obtained from ChemService (West Chester, PA). The purities of all chemical standards were ≥97%. All solvents (Ultra Resi-Analyzed®) were obtained from J.T. Baker (Phillipsburg, NJ).

The spiked food containing a mixture of all the chemicals was made by adding a known quantity of each chemical (dissolved in 300 ml dichloromethane (DCM)) to 150 g of commercial fish food (Zeigler finfish starter, Gardner, PA; 2 mm pellets, 50% protein, 15% lipid, 2% fiber) in a round bottom flask and slowly evaporating the solvent to dryness using a rotary-evaporator. The food was air dried for 24 h and then stored in amber jars at 4 °C. Control food was treated in an identical manner but without the addition of the analytes. The concentrations of each triazole and α-HCH (Table 1) were determined in spiked and control food using the same technique described below for fish tissue. While these concentrations are above those likely to be found in the environment, which have been measured at low part per billion levels (Mortensen et al., 1998), they were required for chemical detection in the fish. Furthermore, the results of this study are applicable regardless of exposure level because first order elimination kinetics (see below) are independent of concentration.