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A comparison of fish pesticide metabolic pathways with those of the rat and goat

Richard C. Kolanczyk*, **Jose A. Serrano**, **Mark A. Tapper**, and **Patricia K. Schmieder**

US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804, USA

Abstract

Ecological risk assessments are often limited in their ability to consider metabolic transformations for fish species due to a lack of data. When these types of evaluations are attempted they are often based on parent chemical only, or by assuming similarity to available mammalian metabolic pathways. The metabolism maps for five pesticides (fluazinam, halauxifen-methyl, kresoxim-methyl, mandestrobin, and tolclofos-methyl) were compared across three species. A rapid and transparent process, utilizing a database of systematically collected information for rat, goat, and fish (bluegill or rainbow trout), and using data evaluation tools in the previously described metabolism pathway software system MetaPath, is presented. The approach demonstrates how comparisons of metabolic maps across species are aided by considering the sample matrix in which metabolites were quantified for each species, differences in analytical methods used to identify metabolites in each study, and the relative amounts of metabolites quantified. By incorporating these considerations, more extensive rat and goat metabolism maps were found to be useful predictors of the more limited metabolism of the five pesticides in fish.

Keywords

Metabolism; Biotransformation; Metabolic Map; Rainbow Trout; Bluegill Sunfish; Rat; Goat; MetaPath; Risk Assessment; Species Extrapolation

1. Introduction

One aspect of species extrapolation for assessing ecological risk that can be relevant both to exposure and effects characterizations is understanding xenobiotic metabolism, both as a means of detoxification as well as bioactivation. Additionally, comparison of metabolism pathways across species serves to provide better understanding where similarities and differences exist in biotransformation reactions that may lead to enhanced toxicity. This can

*Corresponding author. Phone: +1 218 529 5152 FAX: +1 218 529 5003. kolanczyk.rick@epa.gov (R.C. Kolanczyk).

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Conflicts of Interest

All authors declare that they have no competing financial interests.

then serve as a basis for predicting metabolism for an untested species based on known metabolic maps for tested species. Where rules can be formulated for cross-species metabolism based on transformation pathways leading to enhanced toxicity or detoxification, predictions of altered susceptibility due to species differences in metabolism can be used to better evaluate risk where empirical metabolism data is lacking.

Due to data requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for the registration of pesticides, information on metabolic pathways is relatively rich for certain individual pesticides, pesticide classes and certain animal species. For example, for pesticides used on food crops, metabolism data collected following internationally harmonized guidelines is submitted to the United States Environmental Protection Agency (USEPA) by registrants as part of the pesticide registration process. Understanding metabolism across species for pesticide chemicals where the data exists will allow for the extrapolation of concepts and lessons learned to data poor species and chemicals. For example, the same type of systematically collected data is not as available for industrial chemicals, and is limited for most species.

Data submissions for registration or re-registration of pesticides, particularly for chemicals used on food products typically includes the submission of rat metabolism and pharmacokinetic data (<https://www.epa.gov/pesticide-registration/data-requirements-pesticide-registration>). Depending on the use scenario, there may be information required on pesticide residues (parent chemical and metabolites) in plants and/or residues in livestock species. For environmental assessments, in addition to standard toxicity test data submitted on the parent chemical there is typically guideline study data in environmental organisms submitted on environmental degradation processes. Environmental degradates may arise from a series of biotic or abiotic transformations for which there are more than a dozen guidelines specified. The required data submissions again depend on the proposed use of the chemical. However, amongst all this data there is seldom any information on fish tissue residues. Occasionally there will be a bioconcentration factor (BCF) study that includes some fish metabolism data but it is not a routine occurrence. Thus, assumptions have to be made which largely attribute any noted toxicity to the parent pesticide chemical form, or assume that metabolic pathways measured in a rat study will hold for other species, including fish.

An important part of pesticide chemical risk assessment is the identification of residues of concern. When residues of concern are identified they are based largely on rat metabolism, livestock metabolism residues (typically hen and goat), and/or plant metabolism residue studies. The question of how to use this information to predict potential residues or degradates of concern for environmental species is not often addressed. Thus, extrapolations are often based on very conservative assumptions and/or the use of comparative toxicity Quantitative Structure Activity Relationships (QSARs).

Ingestion of feed containing pesticides by farmed fish can lead to the uptake and occurrence of pesticide residues in fish products. The potential for pesticide residues to be passed through the food-chain to consumers has driven the European Union to publish new data requirements for fish as part of the pesticide approval process (European Union 2009,

2013a). A working document on the nature of residues in fish has been prepared to provide guidance in conducting fish metabolism studies to quantify and characterise residues which may occur in the edible tissues of fish exposed to pesticides (European Union 2013b). Following that guidance, Schlechtriem et.al., (2016), have developed a standardized testing procedure to study pesticide metabolism by farmed fish in connection with residues in fish feed. As these required studies are eventually submitted in the regulatory process, they too may be used in the comparison of metabolic pathways between fish and other species.

Studying comparative metabolism across species can start to address how well rat metabolism data can predict metabolites (residues) in livestock and in particular in fish where there are very limited data. Assessing the information for representative chemicals across pesticide chemical classes would provide information useful in: (a) determining when metabolism pathways are conserved between mammals and fish and consistent with environmental degradate patterns (to aid in assessing increased toxicity due to metabolites as well as inform bioaccumulation potential of toxic metabolites); and (b) understanding what residues and degradates can be excluded from a 'residues of concern' calculation thus reducing exposure and risk estimates. Better understanding of metabolic capability across taxa may also help explain differences in vulnerability across taxa. Investigations into cross-species metabolism to help address basic assumptions, and the building of models that predict metabolite formation across species would decrease the uncertainties in risk assessments for ecological species, particularly where metabolism is characterized as a major uncertainty. This paper summarizes metabolism data on five pesticide chemicals from guideline studies for three species: rat, goat and fish, and presents an approach to comparing metabolism across species as a means to detect important similarities or differences in vertebrate metabolism.

2. Methods

Data from pesticide experimental studies are submitted by registrants to various regulatory agencies for assessment and approval as part of the pesticide registration process. Summaries prepared by regulatory agencies of specific experimental data supporting risk assessments for each chemical and guideline type may be presented as a Data Evaluation Record (DER) in the USEPA and Canadian Pest Management Regulatory Agency (PMRA), or as a Draft Assessment Report (DAR) for European submissions under the auspices of the European Food Safety Authority (EFSA). Pesticide metabolism data for rat, goat and fish produced by registrants/submitters following internationally harmonized test guidelines was extracted from study summary reports in the public domain for the work presented here:

1. B.6.1 Metabolism Study – Rat (USEPA OCSP test guideline 870.7485); (PMRA DACO 4.5.9); (OECD guideline 417) http://www.oecd-ilibrary.org/environment/test-no-417-toxicokinetics_9789264070882-en
2. B.7.2 Metabolism, Distribution, and Expression of Residues in Livestock (USEPA OCSP test guideline 860.1300); (PMRA DACO 6.2); (OECD guideline 503) http://www.oecd-ilibrary.org/environment/test-no-503-metabolism-in-livestock_9789264061873-en

3. B.9.2 Bioaccumulation in Fish: Aqueous and Dietary Exposure (USEPA OCSPP test guideline 850.1730); (PMRA DACO 9.5.6); (OECD guideline 305) http://www.oecd-ilibrary.org/environment/test-no-305-bioaccumulation-in-fish-aqueous-and-dietary-exposure_9789264185296-en

Pesticide registrant studies submitted to regulatory authorities are kept as confidential business information until a pesticide is registered and data is released. The pesticide study summaries used in this analysis were found in the public domain. Data may be found in EFSA public versions of DAR's (<http://www.efsa.europa.eu>); from the Food and Agriculture Organizations (FAO) of the United Nations at <http://www.fao.org>; the World Health Organization (WHO) at <http://www.who.int>; and peer reviewed toxicological monographs from the Joint Meeting on Pesticide Residues (JMPR) found at the International Programme on Chemical Safety (IPCS) INCHEM website (<http://www.inchem.org>).

A metabolism pathway tool, MetaPath (Kolanczyk et al, 2012), for building metabolism databases and with efficient and targeted tools for analyzing metabolism data, was used in this project. MetaPath is publicly available through the OECD QSAR Toolbox download (<http://www.oecd.org/env/ehs/risk-assessment/oecd-qsar-toolbox.htm>). Metabolism study summary data for three species, rat, goat, and fish (either bluegill sunfish or rainbow trout) for five pesticide chemicals, fluazinam, halauxifen-methyl, kresoxim-methyl, mandestrobin and tolclofos-methyl were collected from the public domain and added to a MetaPath database. The pesticide parent chemical and the metabolites identified in each study were coded in the database as individual chemical structures with assigned connectivity resulting in the depiction as a map, or pathway, that could be visually compared to metabolic pathways of the same chemical in the other species. The meta-data associated with each study and the generated metabolism map was also captured to understand the origin of the data, the experimental design, chemical, species, and concentrations tested; the sample matrices analyzed; the analytical chemistry techniques used to identify metabolites; references, etc.

Metabolism assays for rat, goat and fish result in different sample matrices that are designed to answer questions specific to the purpose of each guideline. For example, the fish and goat muscle samples reflect the desire to characterize residues found in a food source entering the human food chain while in the rat studies, excreta (urine and feces) are monitored to obtain toxicokinetic information and assist in mass balance.

The rat data entered following guideline study 870.7485 are designed to examine the toxicokinetics of a chemical substance to obtain information on its absorption, distribution, metabolism and excretion in relation to toxicity tests on the same species. The metabolism data provides useful information for assessing the toxicological significance with respect to bioactivation or detoxification attributable to metabolites of the parent chemical. Studies generally include groups of at least four male and female rats (6-12 weeks old), exposed to a ¹⁴C-radiolabeled single high and low concentration of each chemical for 168 hrs. The high dose, selected to be at a level lower than the LD₅₀, and low dose at a fraction of the high dose were administered by oral gavage. Metabolites >5% of administered dose were characterized in excreta and sometimes in bile, blood/plasma, kidney and liver if

appropriate. Any deviations from the typical experimental design and matrices collected for the identification of metabolites is included in the Results presented for each pesticide.

The goat studies designed to detect residues in a lactating ruminant following Test Guideline 860.1300 measure the parent chemical and metabolites for potential transference to consumers through the consumption of edible organs, tissues, and milk. An estimate of total residues found in the edible livestock commodities as well as disposition to the excreta are provided. The dosage used in livestock oral metabolism studies is the approximate level of exposure expected from the feeding of treated crops. A single female goat is orally dosed daily for five days with ^{14}C radiolabeled test chemical. Excreta and milk are sampled twice a day and muscle (loin and flank), liver, kidney and fat taken at sacrifice, 6-12 hours after the last dose. Metabolite level of 0.05 mg/kg or 10% of total radioactive residue is the threshold for identification. Any deviations from the typical experimental design are included in the Results presented for each pesticide.

Metabolic pathway information for fish is relatively limited. Thus a concerted effort was made to procure fish metabolism maps associated with fish bioaccumulation guideline studies (850.1730). If the BCF is to be based on the parent chemical, the major metabolites should be characterized at the end of the uptake phase of the test. Major metabolites are defined as those which represent 10% of the total radioactive residue or 5% at two consecutive sampling points. Studies added to the database were primarily conducted on bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*). The typical bioaccumulation guideline study includes exposure of enough fish of mixed gender sufficient to provide at least four fish when sampling each time point. One or two concentrations of ^{14}C -labeled test chemical in the range of 0.001 to 0.030 mg/L were delivered via water uptake for at least 28 days. The parent chemical and any identified metabolites were measured in two sample matrices, the fillet or edible tissue, which includes body, muscle, skin, skeleton, and the non-edible viscera which includes the head, fins, and internal organs. Again, any deviations from the typical experimental design are included in the Results presented for each pesticide.

A systematic search for available fish metabolism associated with bioaccumulation studies was performed on the USEPA proprietary data repository (Prism Documentum). Fish metabolism studies were then selected for chemicals that also had rat and goat study data readily available. Consideration was given to those pesticides with study summary data available in the public domain as found on the EFSA site containing public versions of study summaries.

3. Results

A process for simplifying species comparisons and focusing on commonalities in metabolic reaction types was developed by examining metabolites found in each species and then making multiple comparisons across species. The analysis was rapidly achieved by use of the following MetaPath data analysis tools; Highlight Treatment Group, General Search Query, Map Comparison, and Map Extraction (Kolanczyk et al, 2012).

3.1. Fluazinam metabolism in the bluegill, rat, and goat

Biotransformation of the pyridine fungicide, fluazinam at 0.001 mg/L water exposure for 28 days, in the bluegill resulted in metabolites formed through reduction of the nitro groups either ortho to the chlorine, MAPA, or para to the chlorine substituent, AMPA [EFSA 2006a; ECHA 2008]. Further reduction of the second nitro group gives rise to the di-amino metabolite DAPA (Fig. 1). No cleavage of the amine linkage between the two ring system of fluazinam occurred.

Urine, feces and bile were examined for metabolites in a rat study conducted with high (50 mg/kg) and low (0.5 mg/kg) oral doses of Fluazinam (EFSA 2006b). Comparison of bluegill and rat fluazinam metabolic maps is shown in Fig. 2. The figure depicts the metabolic map of bluegill on the left and the rat on the right. Structures within green boxes are common to the metabolic maps of both species, while those in red boxes are exclusive to that species, and tan boxes represent phase II conjugates. Metabolites common to both species are AMPA and DAPA. The bluegill metabolite, MAPA, was not found in the rat study and AMPA-mercapturate was not found in the bluegill. Additionally, cysteine and glucuronide conjugates of DAPA were found exclusively in the rat metabolism study.

Female goats were administered Fluazinam at a rate of 9 mg/kg daily for four days to study metabolism (EFSA 2006c). Comparison of the bluegill and goat studies agree in that both species produce the mono-amine AMPA and di-amino DAPA (Fig. 3) found in multiple matrices including muscle. The bluegill study reports the alternate mono-amine structure MAPA which was not found in the goat. The goat study gave rise to exclusive AMPA-isomer which was a major acid hydrolysis product obtained by rearrangement of AMPA under acid conditions. Not found in the bluegill, the goat study also reports sulfamate conjugates of both AMPA and DAPA.

The distribution of fluazinam and metabolites in various matrices across the three species is summarized and shown in Table 1. Presence of metabolite is designated by an X in the respective column. Major metabolic pathways and respective metabolites are represented in the overall metabolic map (Fig. 1) by thicker arrows.

3.2. Halauxifen-methyl metabolism in the bluegill, rat, and goat

The pyridine carboxylic acid ester herbicide, Halauxifen-methyl, undergoes esterase cleavage to the free acid Halauxifen in the bluegill sunfish dosed at 0.002 and 0.02 mg/L water for 28 days (Fig. 4) (EFSA 2013a). The other major route of metabolism is the O-dealkylation of Halauxifen-methyl to the free phenol, X11406790. Both Halauxifen and X11406790 can produce the free acid, free phenol X11449757 via O-dealkylation and de-esterification respectively. Subsequent to these phase I reactions are the formation of numerous phase II O-glucuronide and sulfate conjugates. Additionally, an N-glucuronide conjugate of Halauxifen-methyl was also observed in the bluegill (Fig. 4).

A metabolism and pharmacokinetics study was done on rats dosed orally with 10 mg/kg Halauxifen-methyl (EFSA 2013b). Comparison of bluegill versus rat metabolic maps provided a reasonably good correlation of biotransformation products (Fig. 5). The free acid, Halauxifen and its glucuronide conjugate are found in both species. The O-dealkylated

Halauxifen-methyl (X11406790) was only found in the bluegill map as were the subsequent sulfate and glucuronide conjugates. However, the O-dealkylated free acid (X11449757) was found in both studies. The rat map included sulfate and glucuronide conjugates for X11449757 that were not observed in the bluegill.

Lactating goats were exposed to a 10 mg/kg oral dose of Halauxifen-methyl for four days (EFSA 2013c). A very good correlation was observed when bluegill and goat metabolic maps for Halauxifen-methyl were compared (Fig. 6). Muscle tissue was not specifically measured in goats, therefore the complete maps including all matrices were compared. The free acid Halauxifen, X11406790 O-dealkylation product and the O-dealkylated free acid X11449757 were all found in the maps of both species. Differences between the two pathways revolved around the specific respective conjugates that were observed for each species.

Distribution of Halauxifen-methyl and metabolites in the various matrices across three species is shown in Table 2. Major metabolic pathways are represented in the overall metabolic map for Halauxifen-methyl (Fig. 4) by thicker arrows.

3.3. Tolclofos-methyl metabolism in the bluegill, rat, and goat

Routes of biotransformation in bluegill sunfish of the organothiophosphate fungicide, Tolclofos-methyl (TM) via water exposure at 0.03 mg/L (EFSA 2005a) include oxidative desulfuration to the oxon, TMO (Fig. 7). Cleavage of the phosphate ester of both parent TM and TMO to the phenol (Ph-CH₃) was assumed to have occurred. Hydroxylation of the phenyl-ring methyl group produced the benzyl-alcohol of both TM and TMO as presumed intermediates TMO-CH₂-OH and TM-CH₂-OH. The benzyl alcohol Ph-CH₂-OH resulting from oxidation of Ph-CH₃ was observed as a quantifiable metabolite. Further oxidation of the benzyl-alcohols of both TM and free phenol Ph-CH₃ result in formation of the aldehydes Ph-CHO and TM-CHO. The complete oxidation of Ph-CHO forms the carboxylic acid Ph-COOH. Of the residues found in bluegill tissues, 79-81% in fillet and 39-46% in viscera were attributed to parent chemical.

The metabolism of high (200 mg/kg) and low (5 mg/kg) dose Tolclofos-methyl in the rat resulted in the identification of ten metabolites (Fig. 7) (EFSA 2005b,c). A comparison of bluegill and rat metabolic maps for Tolclofos-methyl was performed using the map comparison feature in MetaPath (Fig. 8). Results of the map comparison are essentially captured in Fig. 7 and depicted as those structures marked with an “F” and/or “R” to denote presence of the metabolite in the respective fish or rat map. Major differences between species include demethylation of the phosphate esters of TM and TMO in the rat, but not bluegill. The aldehydes Ph-CHO and TM-CHO found in the bluegill map were not found or listed as intermediates in the rat map but could perhaps be considered as intermediates in the path from benzyl alcohol to benzoic acid. TMO, found as a quantifiable metabolite in the bluegill was listed as an assumed intermediate in the rat map.

Female goats were administered Tolclofos-methyl at a rate of 10 mg/kg for four days to study metabolism (EFSA 2005d). A comparison of the bluegill and goat metabolic pathways including all matrices resulted in all the fish metabolites to be found in the goat map (Fig. 9).

In the goat as in the fish, formation of TMO was actually observed in contrast to the rat, where it was an assumed intermediate. A matrix comparison between the bluegill fillet and goat muscle could not be attempted as no muscle metabolites were identified in the goat study. Additionally, the aldehydes found in the bluegill, Ph-CHO and TM-CHO, were also found in the goat. As in the rat comparison, demethylation of the phosphate ester was also found in the goat but not the bluegill. A glycine conjugate of Ph-COOH was found in the goat but not in either bluegill or rat.

The three main types of transformation as expected for organothiophosphates; oxidative desulfuration, phosphate de-esterification cleavage, and phosphate ester demethylation of Tolclofos-methyl are demonstrated across the three species examined. The majority of the residue found in the fish was the parent Tolclofos-methyl in both fillet (80%) and viscera (43%). The distribution of Tolclofos-methyl and metabolites in various matrices across three species is shown in Table 3. In contrast, a majority of the radioactivity for both the rat urine and feces and goat sample matrices was found as metabolites rather than parent chemical. More specifically the nature of those metabolites involves the O-demethylation of the methoxyphosphate esters, shown as DM metabolites in Table 3. Further inspection shows the DM metabolites were found in urine which for this chemical was not a good matrix comparison for the fillet or viscera tissue residues of the fish. When considering a more appropriate sample matrix for comparison, liver, kidney, milk and feces of the goat as a composite sample would be close to that which comprises the viscera of the fish. The differences observed in comparing fish to rat or goat might be explained by analysis of sample matrix.

3.4. Kresoxim-methyl metabolism in rainbow trout, rat, and goat

An overall cross-species metabolic map for kresoxim-methyl (BAS 490 F), a strobil fungicide, in rat (R), goat (G), and fish-trout (F) is presented in Fig. 10. For clarity phase II glucuronide and sulfate conjugates were omitted from the scheme. The complete map includes 4 metabolites for fish, 32 for rats, and 9 for goats.

Fish metabolism was measured in association with a bioaccumulation study of kresoxim-methyl in rainbow trout (EFSA 2010a). Trout were exposed to 0.025 mg/L kresoxim-methyl in water for 28 days and the residues found in fillet and viscera tissues were identified. Metabolic pathways observed in the rainbow trout include the direct aromatic ring-hydroxylation of the cresyl-ring of kresoxim-methyl to structure 490M15 (Fig. 10), and the hydrolysis of the methyl ester to form the free acid 490M01, followed by either aromatic ring-hydroxylation of the cresyl-ring (490M09), or alkyl-hydroxylation of the phenylmethyl group (490M02). Of the residues found in rainbow trout tissues, 72-82% in fillet and 40-45% in viscera were attributed to parent chemical.

Metabolism of kresoxim-methyl in the rat dosed at 50 and 500 mg/kg involved a much more extensive and complex pathway involving 32 detected metabolites and six reaction types; (1) cleavage of the ester to 490-M01 (Fig. 10), (2) cleavage of the oxime ether (490-M11), (3) cleavage of the benzyl ether (490-M16; Intermediate 1), (4) aromatic ring hydroxylation of the cresyl-ring (490-M15), (5) alkyl-hydroxylation of the phenylmethyl group (490-M24),

(6) subsequent oxidation of the formed benzyl-alcohol to a carboxylic acid (490-M05) (JMPR 1998a,b; EFSA 2010b,c).

The rainbow trout metabolic map was compared to that submitted for available rat studies utilizing the Map Compare feature within MetaPath to visualize metabolite similarities and differences across metabolite structures. Results of the map comparison are essentially captured in Fig. 10 and depicted as those structures marked with an “F” and/or “R” to denote presence of the metabolite in the respective fish or rat map. The rat map has many additional pathways not found in the fish map such as cleavage of the oxime (490-M11) and benzyl ethers (490-M16, Intermediate 1) and thus does not provide a good correlation when considering absolute numbers of metabolites. Of note is a pathway involving four smaller structures as a result of cleavage reactions, shown on the left side of Fig. 10 labeled as intermediates. These structures were not directly observed in the rat study but their subsequent metabolite or phase II conjugates were. Closer inspection of quantitative data for metabolites identified in the study in rat plasma, liver and kidney (Table 4) indicate the major routes of biotransformation in the rat are cleavage of the ester to the free acid (490-M01) followed by hydroxylation (490-M09) or alkyl-hydroxylation of the phenylmethyl (490-M02). Additional quantitative data for rat excreta found under the table look-up feature in MetaPath (identical to Table 4) support the formation of 490-M15 via aromatic ring-hydroxylation as a predominant pathway. When considering only the major pathways supported by the quantitative data, as shown by heavier arrows which represent >5 to 10% TRR in Fig. 10, biotransformation pathways across rat and trout appear to be well-conserved.

The sample matrix in which metabolites are measured should be considered when drawing comparison between species. The fish metabolism measurements done with bioaccumulation studies generally includes fillet (edible tissue), which includes body, muscle, skin, skeleton, and the viscera (non-edible tissue), which includes the head, fins, and internal organs. In contrast, rat studies typically measure metabolites in excreta, i.e., urine, feces and bile. However, in livestock residue studies with goat, a lactating ruminant, metabolites are measured in edible organs and tissues as well as in milk. Additional map comparisons were performed to determine if metabolites found in more similar sample matrices, goat tissue and fish tissue, are comparable.

The complete metabolic pathway for Kresoxim-methyl dosed at 25 mg/kg/day in the goat (edible organs and tissue and milk compartments) (EFSA 2010d) was compared to that of the rainbow trout (edible and non-edible compartments). All of the fish metabolites are found in the goat map as major pathways (Fig. 11 green boxes). These reactions include cleavage of the methyl ester bond to the free acid (490-M01) followed by either aryl-hydroxylation of the cresyl-ring (490-M09) or alkyl-hydroxylation of the phenylmethyl group (490-M02). In addition, aryl-hydroxylation was observed without cleavage of the ester methyl (490-M15). As with the comparison of rainbow trout and rat maps, the comparison with goat map resulted in differences in minor metabolic pathways. Exclusive to the goat and not found in the trout or rat were transformations including cleavage of the oxime ether (490-M18) and conjugation with glycine (490-M19). Also observed as a minor pathway in

the goat and rat but not fish, was cleavage of the benzyl ether linkage (490-M06) (Figs. 10, 11).

Species comparison were then performed with more closely matched sample matrices. The metabolic maps for rainbow trout fillet and goat muscle were extracted using the Extract/Merge functionality within MetaPath. The resulting kresoxim-methyl trout and goat muscle tissue maps are shown in Fig. 12. The observed metabolites in the well-matched matrix are in agreement for the major metabolic pathways, with only two additional minor metabolites detected in the goat including the glycine conjugate (490-M19) and cleavage of the oxime ether (490-M18).

An examination of the quantitative data to identify the major metabolic routes, and the extraction of metabolites specific to a common sample matrix was achieved by use of the MetaPath table lookup feature 'Result, met.', and the map 'Extract/Merge' and 'Map Compare' tools. Thus, despite the complexity of the overall map in Fig. 10, it was determined that the major metabolites of kresoxim-methyl in rat, goat, and fish are 490-M15, 490-M01, 490-M09 and 490-M02, and that the metabolic transformations for this strobilin compound are well conserved across these species. The distribution of Kresoxim-methyl and metabolites in the various matrices across three species is shown in Table 5.

3.5. Mandestrobin metabolism in the bluegill, rat, and goat

Identification of residues in fillet and viscera tissues of bluegill sunfish exposed to the strobil fungicide, Mandestrobin (Benzyl-14C)S-2200, was done in association with a bioconcentration study (EFSA 2014a). Metabolic pathways observed in the bluegills include alkyl-hydroxylation of either methyl group on the phenoxy ring (2-CH₂-OH-(S2200) and 5-CH₂-OH-(S2200)) and aromatic ring-hydroxylation of the benzyl ring (4-OH-(S2200)) of mandestrobin (Fig. 13). These are the same types of metabolic transformations seen in the rainbow trout with the strobil kresoxim-methyl, and are conserved across species. Although hydroxylated metabolites were not directly observed in the study, the sulfate and glucuronide conjugates of all three products were found in both the fillet (edible) and viscera (non-edible) tissues exposed at high (10 ug/L) and low (1 ug/L) water concentrations of mandestrobin for 28 days (Fig. 14). Identification of sulfate and glucuronide conjugates of the three hydroxylated metabolites was determined by enzymatic hydrolysis. Mandestrobin was stable in water but was rapidly and extensively metabolized in fish. Some minor unidentified metabolites were also reported but the hydroxylations followed by phase II conjugation were the major metabolic pathways described for bluegills.

A dual radiolabel study was conducted on rats with single low (5 mg/kg), single high (1000 mg/kg), and multi-dose (5 mg/kg) oral administration of mandestrobin (EFSA 2014b,c). The proposed hydroxylation products 2-CH₂-OH-, 5-CH₂-OH- and 4-OH-mandestrobin of the fish study were observed in the rat. However, of the possible sulfate and glucuronide conjugates, only the glucuronide of 4-OH was detected in the rat. A comparison of bluegill sunfish (F) versus rat (R) mandestrobin metabolism is depicted in the overall scheme (Fig. 13). As shown in the figure, mandestrobin was more extensively metabolized in the rat than the fish. In comparison to the fish pathways, additional routes and metabolite identification involving further oxidation of hydroxylated parent to 2-COOH-(S2200) and 5-COOH-

(S2200), demethylation to MCBX, and cleavage reaction (De-Xy-(S2200)) are shown. The primary routes of metabolism in the rat were oxidation and subsequent glucuronidation, demethylation with subsequent oxidation, and oxidation with subsequent demethylation.

The metabolism of mandestrobin administered orally at 35 mg/kg/day for four days to lactating goats measured in milk, organs, and tissues in a dual label study proceeds via a series of hydroxylations and oxidations, N-demethylation, O-demethylation, ether hydrolysis and glucuronide conjugation (EFSA 2014d). Hydroxylation of the phenoxy-ring gives 4-OH-, and hydroxylation of the methyl groups on the phenoxy-ring gives 2-CH₂OH- and 5-CH₂OH- mandestrobin as was indirectly observed in the fish. Three of the six fish conjugated metabolites (2-CH₂-OH-gluc, 2-CH₂-OH-sulfate, and 4-OH-gluc) were observed in the goat. The 4-OH-gluc was identified by LC-MS while the 2-CH₂-OH-gluc and 2-CH₂-OH-sulfate were indirectly shown by acid hydrolysis. Additional metabolic pathways found in the goat include the following reactions. Further oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-, 5-COOH- and 5-CA-2-HM-. Hydroxylation also occurs on the N-methyl group, to give 5-CA-NHM and 5-CA-2-HM-NHM. Hydrolysis of the phenoxy ether link yields De-Xy-. N-demethylation of 5-COOH- results in the formation of 5-CA-NDM, while O-demethylation of mandestrobin results in the formation of MCBX. Similar to the rat/bluegill comparison, the bluegill and goat maps for all matrices measured appears to show a large difference in number and types of metabolites (Fig. 15). When comparing muscle only maps (Fig. 16) there is much more agreement, especially when accounting for the single metabolite De-Xy- displayed six times in the goat muscle map. The additional metabolites found in goat muscle are 5-CA-2-HM-, 5-COOH-, 2-COOH-, and De-Xy-.

The analytical methods used for metabolite identification were identical in the rat and goat studies but different from that used in fish, which could be another source of discrepancy when comparing metabolism studies. The rat and goat studies relied on retention-time match to authentic standards using HPLC and additional confirmation with LC-MS. The goat versus rat maps show a large agreement in terms of major pathways and shared common metabolites (Fig. 13). The fish study also utilized HPLC retention-time match but with thin-layer chromatography (TLC) methods in support. The fish study also reported several minor metabolites whose identification and characterization were not attempted which, if identified, may have resulted in confirmation of more similarity in metabolites.

The distribution of Mandestrobin and metabolites in the various matrices across three species is shown in Table 6.

4. Discussion

The three species guideline studies examined here all provide metabolic maps for in vivo xenobiotic exposures. Unlike studies in the open literature, these guideline studies follow a specific protocol which provides more consistency for data comparisons. When comparing metabolic maps across species, several considerations arise. The disposition of metabolites in body compartments and excreta and thus the ability to detect them may also be dependent on the sample matrix analyzed. For instance, in the fish bioaccumulation guideline study the

residues (metabolites) are analyzed in 2 compartments, the edible fish fillet consisting of muscle tissue and the adjoining skin and ribcage, and the non-edible tissue inclusive of head, fins and internal organs. In contrast, the rat studies report metabolites that are eliminated in the urine, feces, and bile. Only occasionally are metabolites identified in plasma or internal organs of the rat. Therefore, the matrix correlation between fish and rat studies is not ideal; the rat guideline studies are meant to emulate what might happen in humans upon exposure to the pesticide while the fish study determines residues present in edible fillets that might be consumed. The goat metabolism studies are designed to model transference of pesticide and metabolites into potential food products. For this lactating ruminant, the milk, muscle, fat, liver, and kidney are typically analyzed for metabolites. In addition, the blood, gastrointestinal tract, bile, urine and feces are sometimes examined for metabolite identification.

Another consideration is the analytical methods employed to determine metabolites in the various matrices. For example, more recent studies utilizing LC-MS/MS analysis could provide identification and quantification of metabolites not previously possible using less exacting TLC methods used in many older studies.

Metabolism of the pyridine fungicide Fluazinam in the bluegill involved an initial reduction of either of the nitro groups to AMPA or MAPA, followed by complete reduction of both to DAPA (Fig. 1). Neither rat or goat map confirmed MAPA as a metabolite but the path through AMPA and DAPA was demonstrated. The exclusive appearance of MAPA in the bluegill would indicate a species difference. The rat and goat studies provided glucuronide, sulfamate, and cysteine conjugates to the overall Fluazinam map. As shown in Table 1, the conjugates found in the rat and goat were found in the excreta, bile and milk of the respective species. Conjugates were found in the goat liver and kidney tissues. As was shown previously in the kresoxim-methyl comparison there appears to be a better sample matrix match between goat muscle and fat with the bluegill fillet and viscera whereby conjugates were not found in the fluazinam exposure.

The esterase cleavage of the pyridine carboxylic acid ester herbicide Halauxifen-methyl to the free-acid Halauxifen was observed in all three species. The other major route of metabolism, the O-dealkylation of Halauxifen-methyl to the free phenol, X11406790, was only directly found in the bluegill and goat studies. The free-acid, phenol structure X11449757 was observed for all three species and could be produced through either the Halauxifen or X11406790 intermediates in the bluegill and goat. However the production of X11449757 was shown to occur exclusively through the Halauxifen path in the rat. Upon consideration of phase II conjugates, both the rat and goat maps contribute towards an overall map and understanding of possible glucuronide and sulfate formation. Together, both the rat and goat maps provide a good estimate of the metabolites that might be expected in the fish.

In the bluegill study with Tolclofos-methyl, the majority of residues recovered were parent chemical. However, two of the three main types of transformations expected for organothiophosphates, an oxidative desulfuration and a phosphate de-esterification cleavage, were demonstrated in all three species, whereas the demethylation of the phosphate ester

was only shown in the rat and goat. Differences in metabolites identified could perhaps also be due to matrices analyzed. It was particularly notable that many metabolites not found in fish were found in rat and goat urine and feces samples, a compartment not collected in residue analyses done in conjunction with fish bioaccumulation studies.

The metabolic pathways observed in the rainbow trout for Kresoxim-methyl include either (1) the direct aromatic ring-hydroxylation of the cresyl-ring to structure 490M15 or (2) hydrolysis of the methyl ester to form the free acid 490M01, followed by either aromatic ring-hydroxylation of the cresyl-ring (490M09) or alkyl-hydroxylation of the phenylmethyl group (490M02) of kresoxim-methyl. Although there were many other minor metabolites established for both rat and goat, the quantitative data showed the major pathways for these species to include the metabolites mentioned above for rainbow trout. Thus, the major metabolites measure in rat and goat maps were shown to be good predictors for the more limited metabolic pathways in the trout. It was also shown that metabolites detected in goat and fish muscle tissue were comparable for this strobilin.

Similarly, alkyl- and ring-hydroxylations also occurred for the other strobilin fungicide examined, Mandestrobin, where again either rat or goat maps could predict the fish metabolites when limited to the major metabolic routes. However, in the fish there was no apparent oxidation of the alcohol groups to the carboxylic acids as seen in both rat and goats. Additional demethylation and cleavage reactions pathways included in the more complex rat and goat maps were not found in fish.

5. Summary

The metabolic pathways from tissue residue measurements associated with fish bioaccumulation studies, were generally represented very well by the major metabolism pathways in the rat and goat biotransformation studies. However, there are some limitations and concerns. The five chemicals compared in this exercise had a Log Kow range of 3.1 to 4.6. Limited data for fish metabolism in pesticides with low Log Kow exists as the associated bioaccumulation studies are generally only initiated with a chemical Log Kow 3. Upon comparison of the fish data to rat and goat, sample matrix appears to be an important consideration. Although most often minor metabolites, many more metabolites are observed in the mammalian maps as compared to fish. Urine (rat and goat), bile (rat), liver (goat) and kidney (goat) matrices are likely to be enriched in metabolites relative to most other tissues such as muscle/fillet (fish). Viscera samples of the fish also contain some of the tissues that tend to concentrate metabolites, however identification of metabolites could be diluted by inclusion of other tissues that do not concentrate metabolites. In most cases, researchers performing these studies are unlikely to try to identify metabolites in a given matrix unless a significant amount of radioactivity (>5%) is present as something other than parent. When considering these limitations, there is uncertainty regarding the true extent of agreement or disagreement in the metabolic disposition of these five compounds in fish, rats, and goats.

The relatively high percentage of parent chemical identified in the tissue residues of fish as compared to rat or goat could be of concern in instances where the parent chemical is more

toxic than metabolites, or where metabolism leading to greater overall elimination of a chemical residue is erroneously assumed.

The metabolic maps portrayed within MetaPath include the ability to apply expertise tags on individual structures to indicate whether it is an assumed intermediate [CIT] not necessarily found in the study or if some aspect of structural interpretation was applied through expert judgement [EXJ]. In addition tags may be affixed to indicate whether a tolerance expression exists [TE] or if that structure is considered a residue of concern [RC] in the risk assessment. For example Kresoxim-methyl and its acid metabolites; 490-M01, 490-M02, and 490-M09 are regulated residues for which tolerances are expressed in plants, livestock and water. Halauxifen, X11449757, and 11406790 are regulated metabolites of Halauxifen-methyl. In these two examples the residues of concern coincide with the major metabolic pathways (thicker arrows) as indicated in Figs. 4 and 10. However the residues of concern is not always exclusively based upon the major routes of metabolism. Pesticide application with respect to food use and residential areas must also be a consideration. For example, Tolclofos-methyl is used primarily as a non-food use seed treatment. The seeds treated with Tolclofos-methyl did not take up the test substance residue and therefore for this application there was no residue of concern or tolerance.

The process presented herein represents a rapid and transparent approach to comparing metabolism across species using a tool built to facilitate the analyses. Future efforts will involve species comparison of more diverse chemicals as well as starting to approach the issue from a chemical class perspective to identify areas where true species differences in metabolism could result in significant uncertainties in risk assessments that are not confounded by differences in the matrix analyzed or analytical detection method used. Once hypotheses of differential metabolism are raised, experimental systems are available to further sort questions of sample matrix or analytical chemistry miss-matches from those of true species differences in metabolic capabilities. These include systems that allow monitoring of all elimination routes in fish (e.g., bile, urine, feces, gill water) as well as in vitro and ex vivo systems optimized for study of fish biotransformation pathways that will allow better characterization of fish metabolism to facilitate these types of species comparisons.

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References

- ECHA 2008. ECHA Proposal for Harmonized Classification and Labelling - Fluazinam summary of: Lenz NR, Huhtanen KL. Uptake, depuration, and bioconcentration and metabolism of (Fluazinam) Carbon-14 IKF-1216 in bluegill sunfish (*Lepomis macrochirus*) under flow through test conditions. ABC Laboratories, Report No. 5311-93-0013-EF-001, GLP1994 Unpublished Study. https://echa.europa.eu/documents/10162/13626/clh_fluazinam_en.pdf
- EFSA 2005a. EFSA Draft Assessment Report (DAR) Public Version. Tolclofos-methyl, January 2005 volume 3, Annex B, B.9 Ecotoxicology – summary of: Yu CC, Guirguis AS. Metabolism of tolclofos-methyl in bluegill sunfish (*Lepomis macrochirus*). Report No. 411968-7. Sumitomo

Chemical Co., Ltd. Report No. QM-61-0029 GLP1986Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2005b. EFSA Draft Assessment Report (DAR) Public Version. Tolclofos-methyl, January 2005 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: KrautterGR, , DownsJ, , HoglanN, , MarshJD, , LawrenceLJ. Metabolism of tolclofos-methyl in the rat. Pharmacology & Toxicology Research Laboratory. Sumitomo Chemical Co., Ltd, Report No. 81-0025.

GLP1988aUnpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2005c. EFSA Draft Assessment Report (DAR) Public Version. Tolclofos-methyl, January 2005 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: KrautterGR, , DownsJ, , HoglanN, , MarshJD, , LawrenceLJ. Metabolism of tolclofos-methyl in the rat. Final report amendment. Pharmacology & Toxicology Research Laboratory. Sumitomo Chemical Co., Report No. 81-0026. Ltd, GLP1988bUnpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2005d. EFSA Draft Assessment Report (DAR) Public Version. Tolclofos-methyl, January 2005 volume 3, Annex B, B.7 Residue Data – summary of: YuCC, , GuirguisAS. Metabolism of 14C tolclofos-methyl in a lactating goat. Report No. 9, Sumitomo Chemical Co., Ltd, Report No. QM-71-0027. GLP1987Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2006a. EFSA Draft Assessment Report (DAR) Public Version July 2006 volume 3, Annex B, B.9 Ecotoxicology – summary of: LentzNR, , HuhtanenKL. Uptake, depuration, and bioconcentration and metabolism of (Fluazinam) Carbon-14IKF-1216 in bluegill sunfish (*Lepomis macrochirus*) under flow through test conditions. Report No. 5311-93-0013-EF-001, GLP1994Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2006b. EFSA Draft Assessment Report (DAR) Public Version July 2006 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: McClanahanR. Study to identify the metabolites of IKF-1216 (fluazinam) in rats. Report No. 5306-92-0191-AM-002. GLP1995Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2006c. EFSA Draft Assessment Report (DAR) Public Version July 2006 volume 3, Annex B, B.7 Residue Data – summary of: ChengT. Nature of the residue of 14C-Fluazinam (IKF-1216) in lactating goats (Part 1: animal dosing, sample collection and radiochemical analysis, Part 2: metabolite identification and characterization. Report No. 5248-92-0116-EF, GLP1993/1994Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2010a. EFSA Draft Assessment Report (DAR) Public Version. Kresoxim-methyl, March, 2010 volume 3, Annex B, B.9 Ecotoxicology – summary of: MayoBC. 14C-BAS-490 F (14C-Reg.No. 242 009): The bioaccumulation and metabolism in rainbow trout. Huntingdon Research Center Ltd; Huntingdon Cambridgeshire PE18 6ES, Report 1994/10725. GLP1994Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2010b. EFSA Draft Assessment Report (DAR) Public Version. Kresoxim-methyl, March, 2010 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: GansG. Study of the biokinetics of [¹⁴C]-Reg. No. 242009 ([¹⁴C]-BAS 490F) in rats. Report No. 1994/10992. GLP1994Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2010c. EFSA Draft Assessment Report (DAR) Public Version. Kresoxim-methyl, March, 2010 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: KohlW. The metabolism of [¹⁴C]-Reg.No.242009 ([¹⁴C]-BAS 490F) in rats. Report No. 1994/10981. GLP1994Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2010d. EFSA Draft Assessment Report (DAR) Public Version. Kresoxim-methyl, March, 2010 volume 3, Annex B, B.7 Residue Data – summary of: GieseU. Dosing of lactating goats with (14C)-LAB 242009. NATEC – Institut fuer naturwissenschaftlich-technische Dienste GmbH, Hamburg, Germany Fed. Rep. BASF, Report No. 1992/10545. GLP1992Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2013a. EFSA Draft Assessment Report (DAR) Public Version. Halauxifen-methyl, December 2013 volume 3, Annex B, B.9 Ecotoxicology – summary of: XDE-729 methyl bioconcentration and metabolism study with bluegill, *lepomis macrochirus*. Dow AgroScience, DAS Report No. 101135, 66001, Accession Number 2009609. GLP2011Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2013b. EFSA Draft Assessment Report (DAR) Public Version. Halauxifen-methyl, December 2013 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: XDE-729 and

XDE-729-methyl: pharmacokinetics and metabolism in F344/DuCrI rats. Dow AgroScience, DAS Report No. 091098, Accession Number 2006542. GLP2010Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2013c. EFSA Draft Assessment Report (DAR) Public Version. Halauxifen-methyl, December 2013 volume 3, Annex B, B.7 Residue Data – summary of: Nature of the residue study in the ruminant with [¹⁴C]-XR-729 methyl ester. Dow AgroScience, DAS Report No. 101389, Accession Number 2010655. GLP2011Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2014a. EFSA Draft Assessment Report (DAR) Public Version. Mandestrobin, January 2014 volume 3, Annex B, B.9 Ecotoxicology – summary of: Flow-through bioconcentration and metabolism study of [¹⁴C]S-2200 with bluegill sunfish (*Lepomis macrochirus*). Sumitomo Chemical Co., Ltd. Report No. ROM-0016, Study No. 13048.6627. GLP2010Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2014b. EFSA Draft Assessment Report (DAR) Public Version. Mandestrobin, January 2014 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: Amended Final Report 1. [¹⁴C]S-2200: absorption, distribution, metabolism and excretion following repeat oral administration to the rat. Sumitomo Chemical Co., Ltd. Report No. ROM-0036. GLP2011Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2014c. EFSA Draft Assessment Report (DAR) Public Version. Mandestrobin, January 2014 volume 3, Annex B, B.6 Toxicology and Metabolism – summary of: Amended Final Report 1. [¹⁴C]S-2200: absorption, distribution, metabolism and excretion following single oral administration to the rat. Sumitomo Chemical Co., Ltd Report No. ROM-0033. GLP2012Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

EFSA 2014d. EFSA Draft Assessment Report (DAR) Public Version. Mandestrobin, January 2014 volume 3, Annex B, B.7 Residue Data – summary of: [¹⁴C]S-2200 – Absorption, distribution, metabolism and excretion following repeated oral administration to the lactating ruminant. Sumitomo Chemical Co., Ltd. Report No. ROM-0039, Report No. 8227546. GLP2012Unpublished Study. <http://dar.efsa.europa.eu/dar-web/provision>

European Union, 2009. European Union Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC; Off J Eur Union L309, 24.11.2009 2009/150 Available: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=EN> [1 December 2017]

European Union, 2013a. European Union Commission Regulation (EU) No. 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market; Off J Eur Union L93, 3.4.2013 2013/184 Available: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0283&from=EN> [1 December 2017]

European Union, 2013b. European Commission Working Document on Nature of Residues in Fish SANCO/11187/2013 Rev. 3 [Online]. Available: https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_app-j.pdf [1 December 2017]

JMPR 1998a. Monograph: 952. Kresoxim-methyl (JMPR Evaluations 1998 Part II Toxicological). *b) Biotransformation*. Peer review summary of: Gans G. Study of the biokinetics of [¹⁴C]-Reg. No. 242009 ([¹⁴C]-BAS 490F) in rats. Unpublished Report No. 94/10992 from BASF Aktiengesellschaft, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Limbergerhof, Germany 1994 <http://www.inchem.org/documents/jmpr/jmpmono/v098pr10.htm>

JMPR 1998b. Monograph: 952. Kresoxim-methyl (JMPR Evaluations 1998 Part II Toxicological). *b) Biotransformation*. Peer review summary of: Kohl W. The metabolism of [¹⁴C]-Reg. No. 242009 ([¹⁴C]-BAS 490F) in rats. Unpublished report No. 94/10981 from BASF Aktiengesellschaft, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Limbergerhof, Germany 1994 <http://www.inchem.org/documents/jmpr/jmpmono/v098pr10.htm>

Kolanczyk RC, et al. MetaPath: An electronic knowledge base for collating, exchanging and analyzing case studies of xenobiotic metabolism. *Regulatory Toxicology and Pharmacology*. 2012; 63:84–96. [PubMed: 22414578]

Schlechtriem C, et al. Development of a regulatory testing procedure to study the metabolism of pesticides in farmed fish. *Pest Management Science*. 2016; 72:362–370. [PubMed: 25800530]

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Fig. 1.

Overall metabolic map for Fluazinam for Rat (R), Goat (G), and Bluegill fish (F). Thicker arrows indicate major metabolites based on quantitative data.

AMPA = 4-Chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)- α,α,α -trifluoro-5-nitro-m-toluidine

MAPA = 2-Chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)- α,α,α -trifluoro-5-nitro-m-toluidine

DAPA = 4-Chloro-2-(3-chloro-5-trifluoromethyl-2-pyridylamino)-5-trifluoromethyl-m-phenylenediamine

Fig. 2. Comparison of Bluegill fish (left) and Rat (right) metabolic maps for Fluazinam within the MetaPath map comparison feature. Structures within green boxes (____) are common in both metabolic maps while those in red boxes (_____) are exclusive to each respective species. Structures bound by tan boxes (.....) are phase II conjugates.

Fig. 3.

Comparison of Bluegill fish (left) and Goat (right) metabolic maps for Fluazinam within the MetaPath map comparison feature. Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (_ _ _) are exclusive to each respective species. Structures bound by tan boxes (.....) are phase II conjugates.

Fig. 4.

Overall metabolic map for Halauxifen-methyl for Rat (R), Goat (G), and Bluegill fish (F).

Thicker arrows indicate major metabolites based on quantitative data.

X11406790 = methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)pyridine-2-carboxylate

X11449757 = 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-pyridine-2-carboxylic acid

Fig. 5.

Comparison of Bluegill fish (left) and Rat (right) metabolic maps for Halauxifen-methyl within the map comparison feature of MetaPath. Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (_____) are exclusive to each respective species. Structures bound by tan boxes (.....) are phase II conjugates.

Fig. 6. Comparison of Bluegill fillet and viscera (left) and Goat multiple matrices (right) metabolic maps for Halauxifen-methyl within the map comparison feature of MetaPath. Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (____) are exclusive to each respective species. Structures bound by tan boxes (.....) are phase II conjugates.

DM-TM-CH₂OH = O-methyl O-hydrogen O-[2,6-dichloro-4-(hydroxymethyl)phenyl]phosphorothioate
DM-TM-COOH = O-methyl O-hydrogen O-(2,6-dichloro-4-carboxyphenyl)phosphorothioate
TM-CH₂OH = O,O-dimethyl O-2,6-dichloro-4-(hydroxymethyl)phenylphosphorothioate
TM-CHO = O,O-dimethyl O-(2,6-dichloro-4-formylphenyl) phosphorothioate
TM-COOH = O,O-dimethyl O-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
ph-CH₃ = 2,6-dichloro-4-methylphenol
Ph-CH₂OH = 3,5-dichloro-4-hydroxybenzyl alcohol
Ph-CHO = 3,5-dichloro-4-hydroxybenzaldehyde
ph-COOH = 3,5-dichloro-4-hydroxybenzoic acid
ph-CO-glycine = 3,5-dichloro-4-hydroxyhippuric acid

Fig. 8.
Comparison of Bluegill fish (left) and Rat (right) metabolic maps for Tolclofos-methyl within the map comparison feature of MetaPath. Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (____) are exclusive to each respective species.

Fig. 9.

Comparison of Bluegill fish (left) and Goat (right) metabolic maps for Tolclofos-methyl within the map comparison feature of MetaPath. Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (____) are exclusive to each respective species.

Fig. 10.

Overall metabolic map for Kresoxim-methyl for Rat (R), Goat (G), and Rainbow Trout fish (F). To maximize visualization of structures Phase II conjugates were left off of the scheme. Thicker arrows indicate major metabolites based on quantitative data.

490-M01 = (2*E*)-(methoxyimino){2-[(2-methylphenoxy)methyl]phenyl}acetic acid

490-M02 = (2*E*)-(2-{[2-(hydroxymethyl)phenoxy]methyl}phenyl)(methoxyimino)acetic acid

490-M04 = 2-({2-[(*E*)-carboxy(methoxyimino)methyl]phenyl}methoxy)benzoic acid

490-M05 = 2-({2-[(1*E*)-*N*,2-dimethoxy-2-oxoethanimidoyl]phenyl}methoxy)benzoic acid

490-M06 = (2*E*)-[2-(hydroxymethyl)phenyl](methoxyimino)acetic acid

490-M08 = (2*E*)-(2-{[4-hydroxy-2-(hydroxymethyl)phenoxy]methyl}phenyl)(methoxyimino)acetic acid

490-M09 = (2*E*)-{2-[(4-hydroxy-2-methylphenoxy)methyl]phenyl}(methoxyimino)acetic acid

490-M11 = (2*E*)-(hydroxyimino)(2-{[2-(hydroxymethyl)phenoxy]methyl}phenyl)acetic acid

490-M12 = 2-({2-[(*E*)-carboxy(hydroxyimino)methyl]phenyl}methoxy)benzoic acid

490-M15 = methyl (2*E*)-{2-[(4-hydroxy-2-methylphenoxy)methyl]phenyl}
(methoxyimino)acetate
490-M16 = 2-[(1*E*)-*N*,2-dimethoxy-2-oxoethanimidoyl]benzoic acid
490-M18 = (2*E*)-(hydroxyimino){2-[(2-methylphenoxy)methyl]phenyl}acetic acid
490-M19 = {[2*E*]-2-(methoxyimino)-2-{2-[(2-
methylphenoxy)methyl]phenyl}acetyl]amino}acetic acid
490-M20 = (4*E*)-4-(methoxyimino)-1,4-dihydro-3*H*-2-benzopyran-3-one
490-M24 = methyl (2*E*)-(2-{[2-(hydroxymethyl)phenoxy]methyl}phenyl)
(methoxyimino)acetate
490-M27 = (2*E*)-{2-[(2-formylphenoxy)methyl]phenyl}(methoxyimino)acetic acid
490-M56 = (2*E*)-(hydroxyimino){2-[(4-hydroxy-2-methylphenoxy)methyl]phenyl}acetic
acid

Fig. 11.

Comparison of metabolic maps for Kresoxim-methyl across species, Rainbow Trout (left) versus Goat (right). Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (_ _ _) are exclusive to each respective species.

Fig. 12.

Comparison of metabolic maps for Kresoxim-methyl across species, Rainbow Trout fillet tissue (left) versus Goat muscle tissue (right). Structures within green boxes (____) are found common in both metabolic maps while those in red boxes (___) are exclusive to each respective species.

Fig. 13.

Overall metabolic map for Mandestrobin for Rat (R), Goat (G), and Bluegill fish (F). For clarity Phase II conjugates were left off of the scheme. Thicker arrows indicate major metabolites based on quantitative data.

MCBX = 2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-hydroxy-*N*-methylacetamide

4-OH-[S2200] = 2-{2-[(4-hydroxy-2,5-dimethylphenoxy)methyl]phenyl}-2-methoxy-*N*-methylacetamide

2-CH₂-OH-[S2200] = 2-(2-{[2-(hydroxymethyl)-5-methylphenoxy]methyl}phenyl)-2-methoxy-*N*-methylacetamide

2-COOH-[S2200] = 2-(2-{[2-(1-methoxy-2-(methylamino)-2-oxoethyl]phenyl)methoxy]-4-methylbenzoic acid

5-CH₂-OH-[S2200] = 2-(2-{[5-(hydroxymethyl)-2-methylphenoxy]methyl}phenyl)-2-methoxy-*N*-methylacetamide

5-COOH-[S2200] = 3-(2-{[2-(1-methoxy-2-(methylamino)-2-oxoethyl]phenyl)methoxy]-4-methylbenzoic acid

De-Xy-[S2200] = 2-[2-(hydroxymethyl)phenyl]-2-methoxy-*N*-methylacetamide
DX-CA-[S2200] = 2-[1-methoxy-2-(methylamino)-2-oxoethyl]benzoic acid
5-CA-2-HM-MCBX = 4-(hydroxymethyl)-3-({2-[1-hydroxy-2-(methylamino)-2-oxoethyl]phenyl}methoxy)benzoic acid
5-CA-2-HM-MCBX-2H = 4-(hydroxymethyl)-3-({2-[1-hydroxy-2-(methylideneamino)-2-oxoethyl]phenyl}methoxy)benzoic acid
5-CA-2-HM-[S2200] = 4-(hydroxymethyl)-3-({2-[1-methoxy-2-(methylamino)-2-oxoethyl]phenyl}methoxy)benzoic acid
5-CA-MCBX-NDM = 3-{{2-(2-amino-1-hydroxy-2-oxoethyl)phenyl}methoxy}-4-methylbenzoic acid
5-CA-[S2200]-NHM = 3-[(2-{2-[(hydroxymethyl)amino]-1-methoxy-2-oxoethyl}phenyl)methoxy]-4-methylbenzoic acid
5-CA-2-HM-[S2200]-NHM = 4-(hydroxymethyl)-3-[(2-{2-[(hydroxymethyl)amino]-1-methoxy-2-oxoethyl}phenyl)methoxy]benzoic acid
5-CA-[S2200]-NDM = 3-{{2-(2-amino-1-methoxy-2-oxoethyl)phenyl}methoxy}-4-methylbenzoic acid

Fig. 14.

Metabolic profile for mandestrobin aqueous exposure to bluegill sunfish as presented in MetaPath. Identified metabolites were the sulfate and glucuronide conjugates of each of three hydroxylated-parent intermediates labeled (within MetaPath) with a green CIT box indicating the metabolite, cited by the study author, was assumed to have been formed prior to conjugation but not necessarily identified in the study. Multiple colored boxes around the phase II metabolites indicate in which sample matrices the metabolites were detected. The sample matrices selected for visualization (fillet and viscera from 1 and 10 ug/L exposures) are shown selected in the pop-up Highlight Treatment Group functionality of MetaPath.

Fig. 15.

Comparison of Bluegill sunfish edible and non-edible tissues (left) and Goat milk, organs and tissue (right) metabolic maps for Mandestrobilin within the MetaPath map comparison feature. Structures within green boxes (____) are found common in both metabolic maps while those structures in red boxes (____) are exclusive to each respective species. Tan boxes (.....) represent phase II conjugates.

Fig. 16. Mandestrobins metabolic maps for Bluegill (left) and Goat muscle tissue (right) using the MetaPath map comparison feature. Structures within green boxes (____) are found common in both metabolic maps while those structures in red boxes (____) are exclusive to each respective species. Tan boxes (.....) represent phase II conjugates.

Table 1

Distribution of Fluazinam and metabolites in the various matrices across three species. (ECHA 2008, EFSA 2006a,b,c)

	Rat			Goat							Bluegill	
	Urine	Feces	Bile	Urine	Bile	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
Fluazinam		X									X	X
AMPA		X				X	X	X	X	X	X	X
MAPA											X	X
DAPA	X	X		X	X	X	X	X	X	X	X	X
DAPA-gluc	X		X									
DAPA-cys		X										
AMPA-mercaptoprate	X		X									
AMPA-sulfamate					X	X	X	X				
DAPA-sulfamate				X	X	X	X	X				

Table 2

Distribution of Halauxifen-methyl and metabolites in the various matrices across three species. (EFSA 2013a,b,c)

	Rat			Goat					Bluegill	
	Urine	Feces	Blood	Urine	Feces	Milk	Liver	Kidney	Fillet	Viscera
Halauxifen-methyl	X	X	X	X	X	X	X	X	X	X
Halauxifen-methyl-N-Gluc				X		X				X
Halauxifen	X	X	X	X	X	X	X	X	X	X
Halauxifen-O-Gluc	X	X	X							X
X11406790				X	X	X	X	X	X	X
X11406790-sulfate				X		X		X		X
X11406790-O-Gluc				X	X	X	X	X		X
X11406790-N-Gluc				X	X	X	X	X		X
X11449757	X	X		X	X	X	X	X	X	
X11449757-sulfate	X		X	X				X		
X11449757-Gluc	X									

Table 3

Distribution of Tolclofos-methyl and metabolites in the various matrices across three species. (EFSA 2005a,b,c,d)

	Rat		Goat						Bluegill		
	Urine	Feces	Urine	Feces	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
Tolclofos-methyl		X		X						X	X
TMO			X		X					X	X
DM-TM	X	X	X								
DM-SM-TM			X								
DM-TMO	X		X				X				
DM-TMO-CH2-OH	X		X	X	X	X	X	X	X		
DM-TMO-COOH	X										
TMO-CH2-OH			X				X				
TMO-CHO				X							X
TMO-COOH	X	X		X			X				
Ph-CH3		X	X								
Ph-CH2OH		X	X			X				X	X
Ph-CHO			X			X				X	X
Ph-COOH	X	X	X		X	X	X			X	X
Ph-CO-glycine			X								
TM-CH2-OH				X							
DM-TM-CH2-OH	X		X								
TM-CHO				X							X
TM-COOH			X	X							
DM-TM-COOH	X		X		X						

Table 4

Metabolite radioactivity in plasma, liver and kidney of rats following oral administration of a single dose of 50 or 500 mg/kg kresoxim-methyl.

	% of tissue activity													
	Plasma						Liver						Kidney	
	50 mg/kg		500 mg/kg		50 mg/kg		500 mg/kg		50 mg/kg		500 mg/kg		50 mg/kg	
Metabolite	M	F	M	F	M	F	M	F	M	F	M	F	M	F
M01	39.4	25.0	33.0	46.9	20.7	24.7	28.6	42.7	14.4	17.0	19.9	31.5		
M02	9.7		7.0	10.7	13.5	14.5	17.9	13.6	12.3	12.6	17.6	14.1		
M04	4.3		2.2	18.6	4.1	7.3	4.1	6	5.1	4.9	5.2	3.4		
M02, M04		14.0												
M06	2.7	0.5	0.4	1.6			2.4		5.3	1.4	4.9	1.3		
M09	17.6	13.4	11.1	15.3	27.6	27.2	25.6	29.6	35.5	35.6	38.3	40.2		
M16 M26 M11, M12	12.3		17.2		13.1	6.7	8.4	4.9	17.5	6.9	18.3	6.8		
M24	3.1	0.2	0.9	0.8										
M26		7.2		6.1										
Unknown		1.9	1.9	0.6	5.8	2.1								

Table 5

Distribution of Kresoxim-methyl and metabolites in the various matrices across three species. (EFSA 2010a,b,c,d)

	Rat						Goat						Rainbow Trout		
	Urine	Feces	Bile	Plasma	Liver	Kidney	Urine	Feces	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
Kresoxim-methyl	X	X						X					X	X	X
490-M11	X			X	X	X									
490-M12	X				X	X									
490-M01	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
490-M15	X	X						X							
490-M16	X			X	X	X									
490-M24	X	X		X											
490-M19							X	X		X		X			
490-M08	X														
490-M09	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
490-M02	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
490-M18							X	X		X		X	X		
490-M05		X													
490-M06	X			X		X	X	X		X	X		X		
490-M20	X														
490-M27															
490-M04	X	X		X	X	X								X	X
490-M56							X	X	X	X	X	X	X		
Intermediate 1-Gluc			X												
Intermediate 2-Gluc			X												
Intermediate 3-Gluc			X												
490-M24-Gluc			X												
490-M15-Gluc			X												
490-M01-Gluc	X														
490-M02-Gluc			X												
490-M08-Gluc			X												

	Rat						Goat						Rainbow Trout		
	Urine	Feces	Bile	Plasma	Liver	Kidney	Urine	Feces	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
490-M09-Gluc	X		X	X	X	X									
490-M11-Gluc			X												
490-M29 (Gluc)			X												
490-M37 (Gluc)	X		X												
490-M39 (Gluc)			X												
490-M22	X														
490-M41	X														
490-M43	X														
490-M42	X														

Table 6

Distribution of Mandestrobien and metabolites in the various matrices across three species. (EFSA 2014a,b,c,d)

	Rat						Goat				Bluegill	
	Urine	Feces	Liver	Kidney	Plasma	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
Mandestrobien		X				X	X	X	X	X	X	X
MCBX	X	X				X	X	X				
4-OH-S2200	X	X				X	X	X				
5-CH2OH-S2200	X	X				X	X	X				
5-COOH-S2200	X	X	X	X	X		X	X		X		
2-CH2OH-S2200	X	X				X	X	X	X	X		
2-COOH-S2200	X	X	X	X	X		X	X	X	X		
De-Xy-S2200	X	X	X	X		X	X	X	X	X		
5-CA-2-HM-S2200	X	X	X	X			X	X				
5-CA-2-HM-MCBX	X	X					X					
5-CA-2-HM-MCBX-2H	X											
DX-CA-S2200	X											
5-CA-2-HM-S2200-NHM	X	X	X	X			X	X				
5-CA-S2200-NHM	X	X	X	X	X	X	X					
5-CA-S2200-NDM	X	X	X	X	X	X	X		X	X		
5-CA-MCBX-NDM	X	X	X	X		X		X		X		
5-COOH-S2200-Gluc	X				X		X					
4-OH-S2200-Gluc	X				X		X	X	X	X	X	
2-COOH-S2200-Gluc	X				X		X					
2-CH2OH-S2200-Sulf							X				X	X
5-CH2OH-S2200-Sulf											X	X
4-OH-S2200-Sulf											X	X
2-CH2OH-S2200-Gluc							X				X	X
5-CH2OH-S2200-Gluc											X	X
De-Xy-S2200-Sulf							X					
De-Xy-S2200-Gluc							X					

	Rat					Goat					Bluegill	
	Urine	Feces	Liver	Kidney	Plasma	Milk	Liver	Kidney	Muscle	Fat	Fillet	Viscera
5-CA-2-HM-MCBX-Gluc							X					
5-CA-2-HM-MCBX-Sulf							X					
5-CA-2-HM-S2200-Gluc							X					
5-CA-S2200-NDM-Gluc							X					