Prediction of Pesticide Toxicity in Midwest Streams

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Abstract
The occurrence of pesticide mixtures is common in stream waters of the United States, and the impact of multiple compounds on aquatic organisms is not well understood. Watershed Regressions for Pesticides (WARP) models were developed to predict Pesticide Toxicity Index (PTI) values in unmonitored streams in the Midwest and are referred to as WARP-PTI models. The PTI is a tool for assessing the relative toxicity of pesticide mixtures to fish, benthic invertebrates, and cladocera in stream water. One hundred stream sites in the Midwest were sampled weekly in May through August 2013, and the highest calculated PTI for each site was used as the WARP-PTI model response variable. Watershed characteristics that represent pesticide sources and transport were used as the WARP-PTI model explanatory variables. Three WARP-PTI models—fish, benthic invertebrates, and cladocera—were developed that include watershed characteristics describing toxicity-weighted agricultural use intensity, land use, agricultural management practices, soil properties, precipitation, and hydrologic properties. The models explained between 41 and 48% of the variability in the measured PTI values. WARP-PTI model evaluation with independent data showed reasonable performance with no clear bias. The models were applied to streams in the Midwest to demonstrate extrapolation for a regional assessment to indicate vulnerable streams and to guide more intensive monitoring.

Core Ideas
• WARP-PTI models predicted PTI at unmonitored streams in the Midwest.
• The taxon-specific models varied based on explanatory variables and model fit.
• Pesticide use estimates were important in the development of these predictive models.

The occurrence of multiple pesticide compounds (mixtures) in stream water is common for watersheds dominated by developed land uses, including agricultural, urban, or mixed land use. Monitoring of streams by the USGS throughout the United States from 1992 through 2001 showed, for example, that two or more pesticides were present more than 90% of the time and five or more pesticides were detected about 70% of the time in stream water from developed watersheds (Gilliom et al., 2006). The occurrence of mixtures in stream water complicates the assessment of their potential effects on aquatic life. One method for evaluating the biological significance of pesticides in stream water is to express the concentration of each pesticide as a fraction of its toxic concentration. If multiple pesticides are present, these toxicity quotients can be summed to express the potential toxicity of the pesticide mixture according to a concentration addition model.

Concentration addition is a simple, widely used model that assumes that toxicity quotients of all pesticides in the mixture are additive. The Pesticide Toxicity Index (PTI) is a concentration addition model that provides a measure of potential toxicity of pesticide mixtures for key indicator taxa—fish, benthic invertebrates, and cladocera (small crustaceans) (Nowell et al., 2014). First developed by Munn and Gilliom (2001) as a tool to perform screening-level assessments of potential effects of pesticide mixtures on freshwater aquatic organisms, the PTI was subsequently updated (Munn et al., 2006) and refined (Nowell et al., 2014). In theory, the concentration addition model (such as PTI) applies to compounds with similar modes of action and dose–response curves. However, several studies of mixture toxicities have shown that the concentration addition method predicted toxicity to within a factor of 2 or 3 of observed toxicity, regardless of the mode of action of the components (Belden et al., 2007; Faust et al., 2003; Warne, 2003). For environmental water samples, which may contain a large number of pesticides with similar, dissimilar, and unknown modes of action, concentration addition appears to be a slightly conservative but broadly
applicable model with a relatively small likelihood of underestimating effects (Backhaus and Faust, 2012; Belden et al., 2007).

The PTI is a screening tool that incorporates the ability to assess multiple pesticides at once, which is how organisms encounter pesticides in a natural environment (Gilliom et al., 2006). The PTI has been useful in screening-level assessments of existing stream water-quality data by identifying areas of potential concern or explaining the incidence of biological effects in the field (Belden et al., 2007; Mize et al., 2008; Riva-Murray et al., 2010; Sprague and Nowell, 2008). The PTI also has the potential to assist with study planning (e.g., site selection for more intensive monitoring) if an approach could be developed to predict PTI values in unmonitored streams. Predictive models of PTI would help resource managers identify those streams having the highest probability of pesticide-related effects. A recent USGS study of stream quality in the Midwest measured dissolved pesticides in stream water at 100 sites weekly for 12 wk of the 2013 growing season. This spatially and temporally intensive dataset provides an opportunity to develop empirical models for predicting PTI values in unmonitored streams.

Watershed Regressions for Pesticides (WARP) models use multiple linear regression to predict concentration statistics for individual pesticides in streams, using pesticide use and other watershed characteristics as explanatory variables (Larson and Gilliom, 2001; Stone et al., 2013). Once developed, these models can be readily applied to estimate pesticide concentrations in unmonitored streams across wide geographical areas, including estimates of uncertainty in predictions (Larson and Gilliom, 2001; Larson et al., 2004; Stone et al., 2013). WARP models also have been used to estimate the probability of exceeding threshold concentrations of concern, such as USEPA Office of Pesticide Programs Aquatic Life Benchmarks (Stone and Gilliom, 2012; Stone et al., 2013).

The same WARP methodology can also be used to predict a pesticide index, like PTI, which represents mixtures in stream water. Watershed characteristics can be used as explanatory variables to predict stream PTI values for three taxonomic groups and the probability of exceeding levels of concern for PTI values. Applying the WARP model development methodology to PTI (WARP-PTI) provides a tool that incorporates pesticide mixtures and predictions of potential pesticide toxicity. Model estimates of PTI extrapolate monitoring results at a limited number of streams to all streams in the particular geographic region and can be used for prioritizing unmonitored streams for more intensive assessment.

The purpose of this paper is to describe WARP-PTI models for fish, benthic invertebrates, and cladocera; to assess model performance; and to evaluate the models using an independent dataset. Pesticides contributing the most to PTI values across the 100 sites for each taxon are identified, and application of the models to streams in the Midwest is used to illustrate how the models can be used to extrapolate monitoring results.

Materials and Methods

Model Development Dataset

The data used to develop the WARP-PTI models were collected by the USGS National Water Quality Program National Water-Quality Assessment Project in collaboration with the USEPA National Rivers and Streams Assessment Program as part of a regional study of stream quality in the Midwest, called the Midwest Stream Quality Assessment (MSQA). The objectives of MSQA were to assess stream water quality, ecology, and habitat and to evaluate the effects of chemical and physical stressors on aquatic organisms in the streams. Evaluating factors within a watershed that affect these stressors and developing predictive tools to aid in water resource management of the Midwest were therefore high priorities of the MSQA (USGS, 2012).

The MSQA sites were selected to represent a range of agricultural, urban, and least developed land uses within the Midwest (USGS, 2012). The study area was based on aggregated Level 3 Ecoregions of the United States and consisted of portions of the following Midwestern states: Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, Ohio, and Wisconsin (USGS, 2012). A total of 100 sites were included in the study area (Fig. 1). The MSQA field crews collected 12 streamwater samples for pesticide analysis over a span of 14 wk from May through August 2013. Sampling frequency was generally every 7 d, with two skipped weeks during the middle of the sampling season. Specific flow conditions were not targeted; samples were collected on a preset schedule. Stream water samples were collected using isokinetic sampling protocols and fluoropolymer sampling equipment and were filtered using a glass-fiber membrane with 0.7-µm openings in the field (USGS, 2015). Processed stream water samples were immediately chilled and shipped for analysis to the USGS National Water Quality Laboratory (NWQL), where concentrations for 227 pesticides were determined by direct aqueous injection with liquid chromatography–tandem mass spectrometry using USGS method O-2437–15 (Sandstrom et al., 2015). The pesticides analyzed with this method were selected based on a rigorous approach considering agricultural use, transport potential, and toxicity (Norman et al., 2012). Pesticide concentration data are available through the National Water Information System (http://waterdata.usgs.gov/usa/nwis/qw). We used MSQA pesticide data to calculate stream water PTI values for each sample, as described below, at the 100 MSQA sites comprising the model development dataset.

Model Evaluation Dataset

WARP-PTI models developed with MSQA data were evaluated with an independent set of pesticide data collected from the USGS National Water Quality Network (NWQN) for rivers and streams. The NWQN consists of 117 sites across the United States, with 87 sites monitored for pesticides. Pesticide sampling is time weighted to capture the growing season in each region where sites are located. In an effort to use data similar to, but independent of, the model development data, NWQN data collected during 2013 were used exclusively in model evaluation. In 2013, MSQA and NWQN water-quality samples used the same NWQL pesticide analytical method, whereas the NWQN data included 50 sites throughout the United States, with 17 sites in the Midwest. The NWQN sites were chosen for model evaluation based on the availability of both pesticide and explanatory variable data for 2013.

Calculation of the Pesticide Toxicity Index

The PTI methodology used in this study is the most recent and expanded version of PTI calculation (Nowell et al., 2014), which incorporates nearly 500 pesticides and experimental and
nonstandard toxicity data (where standard data were not available). Pesticide Toxicity Index values were calculated for each sample for three distinct taxonomic groups (fish, benthic invertebrates, and cladocera). The PTI consisted of the sum of toxicity quotients for all pesticides detected in the sample mixture, with nondetections and compounds without toxicity data treated as zero concentrations. Toxicity quotients are the measured concentrations of individual pesticides divided by their respective toxicity concentrations (TCs). Toxicity concentrations were determined by a thorough review of available toxicity test data and were determined as the median of all toxicity values (i.e., 50% lethal concentration or 50% effect concentration) researched for a given pesticide and taxonomic group (Nowell et al., 2014), making it robust to outliers. Nowell et al. (2014) describes a Median-PTI and a Sensitive-PTI. This paper focuses on Median-PTI, referred to simply as PTI; however, Sensitive-PTI values were calculated, and Sensitive-PTI models were developed. The Sensitive-PTI development data and tables describing the models can be found in Supplemental Tables S1 through S4.

PTIs were calculated for each sample. The highest calculated PTI at each site during the study period for each taxonomic group was used as the response variable for model development and the observed value for model evaluation. The highest calculated PTI value was modeled to represent the most extreme measured condition that biological communities would encounter. Values for PTI were calculated for the 100 MSQA and 50 NWQN sites using the pesticides determined by the NWQL analysis, where toxicity data were available. A complete list of the 227 pesticides analyzed by USGS method O-2437–15 at the time of the study can be found in Supplemental Table S5.

Watershed Characteristics Used as Explanatory Variables

The PTI models were developed through an empirical, multiple-linear regression approach and were developed independently for each taxonomic group. Potential explanatory variables included a large number of variables that represent pesticide use, land use and population, agricultural management practices, soil properties, physical watershed characteristics, precipitation, and hydrologic properties. Each of the explanatory variables in the broad classes stated above could potentially affect pesticide application or transport and were considered in the multiple-linear regression for each of the three models developed. A description of the 30 major explanatory variables considered in this analysis is provided in Supplemental Table S6.

Toxicity-weighted use intensity (TWUI) is a pesticide use explanatory variable that was introduced in this analysis in place of pesticide use intensity. The toxicity-weighted use (TWU) of a given pesticide in a watershed is determined by its mass use (in kg) divided by the TC of that pesticide. The TWUI is the TWU for a watershed divided by the watershed area. The TWU for each watershed was calculated for 2013 specifically by using pesticide use estimates derived from the E Pest-high method developed by Baker and Stone (2014) divided by TCs from Nowell et al. (2014) and summed across all pesticides used. For example, consider two pesticides applied to a watershed in the same mass; however, one pesticide has a TC that is half of the other, meaning twice as potentially toxic to aquatic organisms. The base pesticide use estimate can be a good predictor of pesticide concentrations in the stream (Larson and Gilliom, 2001; Larson et al., 2004; Stone et al., 2013), but it does not take into
account the TC and would be a poor potential predictor of PTI, whereas TWUI incorporates this difference in TC and the likely response in PTI.

Principal component analysis initially was used to explore potential relations between explanatory variables. Individual soil, hydrologic properties, and precipitation variables often are highly collinear within their groups, and their use individually has the potential to cloud important relations between explanatory variables and PTI. These variables were combined into principal components (PCs) and the resulting PCs added to the list of explanatory variables for consideration in the multiple-linear regression, with the goal of determining if a single principal component contained more explanatory power than the individual variables. Although none of the PC-derived variables was retained in the final WARP-PTI models, this analysis assisted with evaluating potential multicollinearity issues between variables during model development.

**Watershed Regressions for Pesticides Methodology**

The PTI models were developed using multiple-linear regression (MLR), which makes some basic assumptions, including that the variance of the residuals (the difference between the observed and predicted values of the response variable) be constant and that the residuals be independent and normally distributed (Helsel and Hirsch, 2002). When these requirements are not met, predictions based on MLR can be misleading and inappropriate. To rectify possible violations of these assumptions, both response and explanatory variables can be transformed (Neter et al., 1985). Logarithmic, square-root, and fourth-root transformations and the untransformed value were investigated for each possible explanatory variable (see Supplemental Table S6 for a selected list of explanatory variables). The response variable (PTI) was logarithmically transformed. Due to the logarithmic transformation of PTI, values predicted by the logarithmic models (after retransformation) are of the median value expected for each model for a given set of explanatory values rather than the mean value (Helsel and Hirsch, 2002). To obtain an estimate of the mean value, an adjustment for transformation bias is needed (Bradu and Mundlak, 1970; Duan, 1983). However, predicted PTI values were not adjusted for transformation bias because estimates of median values of the models were considered appropriate for the purposes of this study.

Many explanatory variables were considered for inclusion in the models, and it was necessary to narrow the list of variables markedly. The selection of explanatory variables for the MLR models began with the stepAIC procedure (Venables and Ripley, 1999) executed in R with the MASS package. This procedure balances model goodness of fit with the number of parameters needed to achieve that fit by using Akaike’s Information Criterion (Akaike, 1974) and thus seeks to find the model in which the fewest explanatory variables explains the most variation in the response variable.

Explanatory variables were divided into groups based on their variable type (such as soil properties or land use), and stepAIC was implemented to identify the most powerful explanatory variables in each group. The selected variables were first analyzed as a single group with the stepAIC procedure and then a subsampling routine. The subsampling routine randomly chose 50 observations and performed stepAIC on this random subset and then repeated the procedure 100 times. This process allowed for narrowing the large number of explanatory variables to a reasonable set for further evaluation.

Further explanatory variable selection for the three models included evaluations of multicollinearity and normality assumptions and application of scientific judgment. Variance inflation factors were calculated for final model variables to assess for multicollinearity (Helsel and Hirsch, 2002). Explanatory variables that had a high variance inflation factor and were correlated were added to the model one at a time to determine the most useful of the covariates. Normality was then assessed for the residuals of each model. At this point, existing models were evaluated with a routine that added each explanatory variable not used in the model back into the analysis and evaluated for the factors stated above to ensure that the model could not be substantially improved with the addition of another variable. Finally, potential models were subjectively evaluated for reasonableness (for example, the models predict increasing PTI with increasing toxicity-weighted use intensity) and their overall contribution to explaining the variation in the PTI values. Pesticide Toxicity Index values calculated for model development and model evaluation sites and explanatory variables for model development can be found in Supplemental Tables S7 and S8.

**Analysis of Model Fit**

Goodness of fit was analyzed for the models by examining the residual standard error (RSE) and $R^2$. Additionally, the leverage statistic, Studentized residuals, Cook’s D, and DFFITS were evaluated to assess leverage and influence of individual observations (Helsel and Hirsch, 2002). Qualitative assessments of model uncertainty and residual errors were evaluated with box-and-whisker plots (Tukey, 1977) and normality plots.

**Application of WARP-PTI to Streams in the Midwest**

The prediction of a PTI value has associated uncertainty, and this uncertainty can be expressed in terms of a prediction interval for a specified confidence level, which was calculated at the 95th percentile (Supplemental Fig. S1). The probability that a PTI would exceed a numerical threshold was also calculated. The probability of exceeding a threshold takes into account the model error; when the residual error of a model is normally distributed, the error distribution of a predicted value follows a Student’s $t$ distribution. By understanding the error associated with a predicted value, it is possible to estimate the probability that the value exceeds a numerical threshold (Nowell et al., 2006). Prediction intervals and the probability that a predicted PTI will exceed a threshold were calculated by using methods described in Stone and Gilliom (2012).

The WARP-PTI models for fish, benthic invertebrates, and cladocera were used to predict PTI and the probability of exceeding a threshold PTI in unmonitored midwestern streams defined by the EPA River Reach File 1 (RF1) (USGS, 2002) for the Midwest. For each watershed in the RF1, values of explanatory variables were calculated by using geospatial tools, and these variables were used in the three taxon-specific models to make PTI predictions for streams in the Midwest and to compute the probability that those predicted values exceeded a PTI threshold. The PTI thresholds selected for this analysis were 0.01 and 0.1, above which survival rates in
ambient toxicity tests with cladocera were observed to decrease substantially (Newell et al., 2014). The purpose of using two thresholds is to emphasize the relative assessment of toxicity levels.

Results and Discussion

The WARP-PTI models use the explanatory variables found in Table 1. More detail on these explanatory variables can be found in Supplemental Table S6. The WARP-PTI model statistics are shown in Table 2.

The TWUI was the only significant explanatory variable common to all three taxon-specific WARP-PTI models, and the coefficient was positive in all the models. Furthermore, TWUI was the only variable that directly represented a pesticide source term. Land-use–related variables can be considered as surrogate or indirect source-related variables. The URBAN term represents a surrogate for nonagricultural pesticide applications, for which quantitative use estimates are not available. The IRRIG (management practice) term supplies additional water to soil surfaces and can affect runoff to streams, thus reflecting the potential for pesticide transport. The use of SAND (soil property) as an explanatory variable likely reflects that watersheds with a higher percentage of sandy soils have more infiltration and less surface runoff than watersheds with a lower percentage of sandy soils. Although the significant explanatory variables varied between the three taxon-specific WARP-PTI models, in addition to TWUI, the fish and cladoceran models share IRRIG and the benthic invertebrate and cladoceran models share URBAN and SAND as significant predictors of PTI.

Fish WARP-PTI Model

In addition to TWUI and IRRIG (both positive coefficients), FOREST was selected in the fish model with a negative coefficient. Areas with high FOREST are more likely to have fewer pesticides applied than agricultural and urban areas. The fish WARP-PTI model also included available water content (AWC) and base flow index (BFI) as significant explanatory variables (Table 2). The coefficient for AWC (soil property) was positive and likely reflects potential pesticide transport to surface water. Soils with a high percentage of silt and clay have higher AWC than sandy soils (USDA, 1998). Silt and clay soils have a higher potential for runoff than sandy soils, which have more infiltration capacity. In contrast, the coefficient for BFI (hydrologic property) was negative. Streams with high BFI have a higher percentage of flow from groundwater than from surface water, which may mean less potential runoff from soil surfaces.

Benthic Invertebrate WARP-PTI Model

The WARP-PTI model for benthic invertebrates includes TWUI and URBAN (both positive coefficients), SAND (negative coefficient), and SOILCD as significant explanatory variables (Table 2). The coefficient for SOILCD (soil property) was positive; watersheds with more poorly drained soils, like soils in Hydrologic Group C and D, tend to have more surface runoff than infiltration, which may facilitate pesticide transport to streams.

Cladocera WARP-PTI Model

The explanatory variables of TWUI, URBAN, IRRIG, and SAND are included in the cladoceran WARP-PTI model with the same sign coefficients as in the fish and benthic invertebrate WARP-PTI models. The cladoceran model also included PMAY, for which the coefficient was positive (Table 2). Precipitation acts similarly to irrigation by facilitating transport of pesticides to surface waters by supplying water to soil surfaces. Increased precipitation during May, the first month of stream-water sampling, likely facilitated transport of pesticides important to the cladoceran PTI through surface runoff to streams. Precipitation during other months of the sampling season may also transport pesticides but were found not to be significant for this study.

Model Performance

The three taxon-specific WARP-PTI models vary in goodness of fit; however, the models neither consistently underpredicted nor overpredicted PTI values in the model development dataset (Fig. 2; Supplemental Fig. S2). The fish WARP-PTI model had the smallest error (RSE, 0.41), resulting in the highest percentage of sites within a factor of 10 from the observed value (Supplemental Table S9). The cladoceran WARP-PTI model

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Table 2. Statistics and coefficients for the three taxon-specific Watershed Regressions for Pesticides for the Pesticide Toxicity Index models

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept</th>
<th>FOREST</th>
<th>log_{10} URBAN</th>
<th>(TWUI)^{1/4}</th>
<th>(IRRIG)^{1/2}</th>
<th>AWC</th>
<th>log_{10} SAND</th>
<th>SOILCD</th>
<th>PMAY</th>
<th>BFI</th>
<th>R^2</th>
<th>RSE‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>−3.71</td>
<td>0.632</td>
<td>−0.014</td>
<td>na</td>
<td>0.101</td>
<td>6.57</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>−0.017</td>
<td>0.48</td>
<td>0.41</td>
</tr>
<tr>
<td>Benthic invertebrate</td>
<td>−3.817</td>
<td>0.663</td>
<td>na</td>
<td>1.18</td>
<td>na</td>
<td>na</td>
<td>−0.590</td>
<td>0.010</td>
<td>na</td>
<td>na</td>
<td>0.48</td>
<td>0.58</td>
</tr>
<tr>
<td>Cladocera</td>
<td>−3.95</td>
<td>0.794</td>
<td>na</td>
<td>1.17</td>
<td>0.486</td>
<td>na</td>
<td>−0.945</td>
<td>0.004</td>
<td>na</td>
<td>na</td>
<td>0.45</td>
<td>0.68</td>
</tr>
</tbody>
</table>

† Variables are defined in Table 1.
‡ Residual standard error.
had the largest error (RSE, 0.68), corresponding to the smallest percentage of sites within a factor of 10 from the observed value.

Some of the differences in significant explanatory variables between models as well as the differences in model performance may be related to the different pesticides that contributed the most to the highest calculated PTI values. Top contributors are defined as the pesticides that have the highest toxicity quotients for each PTI value, as determined by the concentration of the pesticide and its TC. The top three contributing pesticides to the highest calculated fish PTI at the MSQA sites were the herbicides acetochlor (2-chloro-N-ethoxymethyl-6′-ethylacet-o-toluidide) and metolachlor (2-chloro-N-(6-ethyl-o-tolyl)-N-[(1RS)-2-methoxy-1-methylethyl]acetamide), and the fungicide pyraclostrobin (methyl 2-[1-(4-chlorophenyl) pyrazol-3-yl]oxymethyl]-N-methoxyacetanilate). At 46% of the sites, the top contributors to the highest calculated fish PTI values were acetochlor and metolachlor (Table 3). Each of the three top contributors to the highest calculated fish PTI values are primarily used in agricultural settings, where pesticide use estimates exist.

In contrast, insecticides contributed the most to the highest calculated benthic invertebrate and cladoceran PTI values at the MSQA sites (Table 3). Imidacloprid ([E]-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) was the largest contributor to the benthic invertebrate PTI at 71% of MSQA sites. For cladoceran PTI, the top contributors were split among three organophosphate pesticides (Table 3), of which two had estimated agricultural use in the MSQA study area: tebupirimfos, ([RS]-O-(2-tert-butylpyrimidin-5-yl) O-ethyl O-isopropyl phosphorothioate] and chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate). There were no agricultural use estimates for diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) in the MSQA study area during 2013.

The top contributors to the highest calculated PTI values are important to identify because some aspects concerning the fit of the models can be attributed to their individual use estimates. The lack of an accurate pesticide source term for the top contributors can increase error in the PTI predictions. One of the top contributors to the highest calculated PTI for cladocera (diazinon) did not have agricultural use estimates for the study period and region. Insecticides, like imidacloprid, may have significant nonagricultural use, which was not quantified in the TWUI estimates. Overall, there were 33 unique pesticides or pesticide degradates contributing the most to the highest calculated PTI values across the three taxa and there were no agricultural pesticide use estimates for 18 (55%) of those pesticides or degradates. Quantitative estimates of nonagricultural pesticide use have the potential to improve the WARP-PTI models; however, such data are not available for the Midwest. Urban use estimates are also unavailable; however, the extent of developed land use in a watershed was a surrogate for urban pesticide use, which is included in both the benthic invertebrate and cladocera WARP-PTI models. Additionally, an increased number of smaller contributors, such as is the case with cladoceran PTI, make it more difficult to identify strongly related explanatory variables because each smaller contributor has potential varied application and transport properties.
Table 3. Top three contributing pesticides to the highest calculated Pesticide Toxicity Index values at each of the Midwest Stream Quality Assessment sites for each taxa and the number of sites where each pesticide was the top contributor.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Fish</th>
<th>Number of sites</th>
<th>Benthic invertebrates</th>
<th>Number of sites</th>
<th>Cladocera</th>
<th>Number of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetochlor (H†)</td>
<td>36</td>
<td>36</td>
<td>Imidacloprid (H)</td>
<td>71</td>
<td>Tebupirimfos (I)</td>
<td>23</td>
</tr>
<tr>
<td>Pyraclostrobin (F§)</td>
<td>26</td>
<td>26</td>
<td>Chlorpyrifos (I)</td>
<td>9</td>
<td>Chlorpyrifos (I)</td>
<td>14</td>
</tr>
<tr>
<td>Metolachlor (H)</td>
<td>10</td>
<td>10</td>
<td>Bifenthrin (I)</td>
<td>3</td>
<td>Diazinon (I)</td>
<td>11</td>
</tr>
</tbody>
</table>

† Herbicide.
‡ Insecticide.
§ Fungicide.

Model Evaluation

The three taxon-specific WARP-PTI models were developed with data collected at MSQA sites during May through August 2013. The MSQA sites fit into a specific range of physical characteristics, including geographic region and watershed size. Because the models were developed for these sites and characteristics, the model evaluation step purposefully included sites in other geographic regions, with other watershed characteristics to evaluate applicability outside the MSQA study area and outside the range of MSQA site characteristics. The WARP-PTI models were evaluated with data from the USGS collected in May through August 2013. Figure 2 shows the WARP-PTI model performance with the NWQN model evaluation data and highlights the model evaluation sites in the Midwest, which are independent data that most closely resemble the development data in terms of geographic region. NWQN site PTI predictions in the Midwest, in general, fell more often within a factor of 10 of the observed PTI compared with other sites in the NWQN, but there were a limited number of Midwest NWQN sites (17) (Supplemental Table S9). Even though the model evaluation data differ from the model development data in geographic region and watershed size, the three WARP-PTI models appear to perform with a reasonable amount of utility with the model evaluation data (Fig. 2; Supplemental Table S9).

Model Application to Unmonitored Streams in the Midwest

The three taxon-specific WARP-PTI models were applied to RF1 streams in the Midwest. Figure 3 shows the probabilities of exceeding PTI values of 0.01 and 0.1 for fish (Fig. 3A), benthic invertebrates (Fig. 3B), and cladocera (Fig. 3C). This model application shows vulnerable streams in the Midwest and can be used to guide more intensive monitoring (Fig. 3). Some areas not modeled in the application, for example the area in southeastern Nebraska, had values for one or more explanatory variables that were outside the range of those used in model development. Few stream reaches in the Midwest had more than a 50% likelihood of exceeding a PTI threshold of 0.1 for any taxonomic group. Within the Midwest, the model application indicated that cladocera were more vulnerable than either benthic invertebrates or fish; 0.6% of the 11,781 RF1 stream reaches in the Midwest had greater than a 50% likelihood of the maximum predicted PTI value for cladocera exceeding the PTI threshold of 0.1, compared with 0.03% of stream reaches for benthic invertebrates and no stream reaches for fish. When the threshold is adjusted to a PTI value of 0.01, the percentage of stream reaches with a greater than 50% likelihood of exceedance increased to 0.03, 9.7, and 17.6% for fish, benthic invertebrates, and cladocera, respectively. The model application (Fig. 3) illustrates areas of the Midwest that might benefit from targeted monitoring based on a higher probability of exceeding a PTI threshold.

Model Limitations

The TWUI used for model evaluation was limited to the pesticides analyzed by NWQL method O-2437–15 to be consistent with the WARP-PTI model development (Supplemental Table S5). Use of a TWUI that takes into account additional pesticides would lead to the overprediction of PTI values. The three taxon-specific WARP-PTI models were developed with data collected from MSQA sites during 2013. Every year represents a unique pesticide use pattern in terms of the pesticides that were available and actively applied during that growing season. Uncertainty in application of the WARP-PTI models beyond 2013 is not known; however, it is expected that the models can be used to guide more intensive monitoring for years with a similar suite of pesticides in use. The applicability of the WARP-PTI models in the future will diminish as pesticides are discontinued and new pesticides are introduced. In particular, major changes in the use of those pesticides that were important contributors to PTI in these models will most likely increase uncertainty in these model predictions. Application of these models to watersheds with characteristics outside the range of those used in model development will also result in increased uncertainty.

The observed PTI value used as the response variable in model development was the highest calculated PTI for each site over the MSQA study period. Given the MSQA sampling design (weekly samples without storm sampling), it is probable that the actual maximum PTI value occurring within the study period was greater than the highest calculated PTI value for each site during the same period. Therefore, the WARP-PTI models likely underpredict the actual maximum PTI values that occurred at the model development sites. Another factor that leads to underestimation of PTI values was the assumption that censored values were equal to zero concentrations. The TWUI variable was limited to agricultural-use intensity because data for nonagricultural pesticide use were not available. Application of these models to predict PTI driven by pesticides with significant nonagricultural use may result in increased uncertainty and potentially biased results.
Conclusions

Most assessments and modeling of potential pesticide concentrations in streams are focused on individual pesticides; however, aquatic organisms are often exposed to multiple pesticides concurrently in streams. The PTI, a tool for assessing mixtures of pesticides in stream water, was combined with a WARP modeling approach to develop empirical multiple linear regression models that can be used to predict PTI values in unmonitored streams in the Midwest. These predictions are an expansion on the traditional WARP models and incorporate a new explanatory variable, toxicity-weighted use intensity. The WARP-PTI models can guide the targeting of more intensive biological assessment with the advantage of incorporating the potential effects of pesticide mixtures.

References
