Hazard/Risk Assessment

APPROACHES FOR LINKING WHOLE-BODY FISH TISSUE RESIDUES OF MERCURY OR DDT TO BIOLOGICAL EFFECTS THRESHOLDS

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Abstract—A variety of methods have been used by numerous investigators attempting to link tissue concentrations with observed adverse biological effects. This paper is the first to evaluate in a systematic way different approaches for deriving protective (i.e., unlikely to have adverse effects) tissue residue-effect concentrations in fish using the same datasets. Guidelines for screening papers and a set of decision rules were formulated to provide guidance on selecting studies and obtaining data in a consistent manner. Paired no-effect (NER) and low-effect (LER) whole-body residue concentrations in fish were identified for mercury and DDT from the published literature. Four analytical approaches of increasing complexity were evaluated for deriving protective tissue residues. The four methods were: Simple ranking, empirical percentile, tissue threshold-effect level (t-TEL), and cumulative distribution function (CDF). The CDF approach did not yield reasonable tissue residue thresholds based on comparisons to synoptic control concentrations. Of the four methods evaluated, the t-TEL approach best represented the underlying data. A whole-body mercury t-TEL of 0.2 mg/kg wet weight, based largely on sublethal endpoints (growth, reproduction, development, behavior), was calculated to be protective of juvenile and adult fish. For DDT, protective whole-body concentrations of 0.6 mg/kg wet weight in juvenile and adult fish, and 0.7 mg/kg wet weight for early life-stage fish were calculated. However, these DDT concentrations are considered provisional for reasons discussed in this paper (e.g., paucity of sublethal studies).

Keywords—Mercury  DDT  Residue effects  Critical body burden  Threshold effects

INTRODUCTION

Numerous investigators have reported experimental results linking tissue residues and biological effects for a variety of bioaccumulative environmental contaminants and aquatic biota [1—9]. McCarty and Mackay [10] argued that identifying critical body residues is a more direct measure of toxicological dose-response than linking biological effects to external media levels (e.g., water, sediment concentrations). The U.S. Environmental Protection Agency (U.S. EPA) supports this residue approach and has been moving toward using tissue concentrations for deriving ambient water-quality criteria for bioaccumulative environmental contaminants (http://www.epa.gov/waterscience/criteria/aleg_sab.draft.pdf).

Three compilations of published literature summarize experimental results reporting biological effects and associated tissue residues in aquatic organisms ([11,12]; Environmental Residue Effects Database [http://www.wes.army.mil/el/ered/]). These compilations generally focused on biologically important endpoints (e.g., survival, growth, reproduction) and corresponding whole-body or organ-specific tissue concentrations. The compilations did not make assumptions regarding toxic modes of action or identify critical body residues associated with adverse effects. Attempts to derive protective tissue concentrations for use in risk assessment have increased with the publication of these residue compilations. However, a critical evaluation of approaches for data treatment has not yet been reported.

This study reviewed existing residue-effects publications dealing with mercury and DDT and obtained information using consistent decision rules developed herein. We evaluated four approaches for analyzing data, and identified whole-body tissue concentrations of mercury and DDT that are protective of fish. In this paper, we use the term protective to mean concentrations below which adverse effects in most fish are unlikely. The U.S. EPA has set as a major goal identifying protective levels when assessing ecological risks at Superfund sites [13] and establishing water quality criteria [14]. In ecological risk assessments, protective levels are derived from the threshold for adverse effects, i.e., that area of the dose-response curve in the vicinity of the no-observed-adverse-effect level to the lowest-observed-adverse-effect level. We selected mercury and DDT to evaluate in this study because they are persistent, bioaccumulative, and toxic contaminants detected routinely in fish at hazardous waste sites as well as throughout the United States [15]. Thus, our findings may have broad application to the research community as well as to environmental managers.

In establishing water-quality criteria, the U.S. EPA uses a preponderance of the evidence (i.e., toxicological investigations meeting minimum data quality requirements and involving multiple test organisms and endpoints) and careful statistical analysis to develop numerical water concentrations that are protective of aquatic organisms [16]. Similarly, sediment quality guidelines have been developed using a preponderance of evidence to derive threshold concentrations (e.g., [17,18]). More recently, probabilistic methods, such as species-sensitivity distributions, have been extended to risk assessment...
corresponding chemical tissue residues associated with no and exposed, chemical form, exposure scenario, biological response were included if the author(s) reported effects correlated with than one chemical (e.g., hatchery spawning studies), results used single chemical exposures. For studies involving more exclusions because the threshold for adversity was omitted based on very poor laboratory control survival (47%) [21]. Papers reporting only effects concentrations were not acute for species or life stage.

Prior to data analysis, the paper was processed using a set of decision rules to evaluate the acceptance of the paper and to select the appropriate residue-effects information from each publication. These decision rules reflect our goal to develop protective levels for mercury and DDT in fish. The terms no-effect residue (NER) and low-effect residue (LER) are used in Table 2 and throughout this paper to bound and identify the threshold for adverse effects within each paper. The NER (analogous to the no-observed-adverse effect level) is defined as the highest chemical concentration in the organism’s whole body, below which adverse effects are not observed or rare. The LER (analogous to the lowest-observed-adverse-effect level) is the lowest chemical concentration in the organism’s whole body associated with an increasing incidence of adverse effects.

METHODS

Literature review and data selection

Published papers reporting mercury or DDT residues in fresh- or saltwater fish and associated biological responses were identified from published database compilations ([11,12]; U.S. Army Corps of Engineers Environmental Residue-Effects Database [http://www.wes.army.mil/el/ered]), electronic library literature searches (e.g., ISI Web of Knowledge, Aquatic Science & Fisheries Abstracts), and comprehensive bibliographies (e.g., U.S. Geological Survey CERC Publications database [http://www.cerc.usgs.gov/pubs/center/pdfDocs/CercPubs.pdf]). Published investigations vary widely in design, execution, and data presentation. To help bring uniformity to the treatment and analysis of this published residue-effect information, we developed discrete guidelines and decision rules for reviewing papers and obtaining residue-effects information in a consistent manner (Tables 1 and 2). Papers reporting no-effect concentrations exclusively or studies using subcutaneous injection as the exposure route were excluded a priori. The remaining published reports were evaluated further using all the guidelines in Table 1. For example, one paper was omitted based on very poor laboratory control survival (47%) [21]. Papers reporting only effects concentrations were excluded from the data analysis because the threshold for adverse effect could not be bounded. Almost all papers reviewed used single chemical exposures. For studies involving more than one chemical (e.g., hatchery spawning studies), results were included if the author(s) reported effects correlated with a single chemical or concluded that a single chemical likely was responsible for observed effects.

The following information was selected from each paper meeting the Table 1 guidelines: Fish species and life stage(s) exposed, chemical form, exposure scenario, biological response examined, control or reference concentration, and the corresponding chemical tissue residues associated with no and low effects. The latter information especially is critical because it characterizes that area of the dose-response curve associated with the threshold for adverse effects. Selecting this information from each paper often was problematic. For example, some investigations reported variable tissue residues over time and/or among replicates even when exposure concentrations were relatively constant. Therefore, a set of decision rules (Table 2) was developed to obtain consistently acceptable threshold residue-effects information from each paper. These decision rules reflect our goal to develop protective levels for mercury and DDT in. The terms no-effect residue (NER) and low-effect residue (LER) are used in Table 2 and throughout this paper to bound and identify the threshold for adverse effects within each paper. The NER (analogous to the no-observed-adverse effect level) is defined as the highest chemical concentration in the organism’s whole body, below which adverse effects are not observed or rare. The LER (analogous to the lowest-observed-adverse-effect level) is the lowest chemical concentration in the organism’s whole body associated with an increasing incidence of adverse effects (dose- or threshold-response). A statistically significant difference was not a requirement for using published data, but was reported for almost every study.

Except where noted, the term mercury refers to total mercury, the expression most commonly used in the studies we reviewed. The term DDT refers to the sum of DDT, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyl dichloroethylene (DDD) and their isomeric forms. When individual DDT compounds or isomers were reported, we calculated and reported the sum. All tissue concentrations in this paper are expressed as mg/kg wet weight.

Data analyses

Four analytical approaches of increasing complexity were evaluated for deriving protective levels of mercury and DDT

<table>
<thead>
<tr>
<th>Table 1. Characteristics required for accepting mercury and DDT tissue residue-effects literature</th>
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<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Bounded effect and no-effect concentrations</td>
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<td>Control/reference treatments</td>
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<td>Whole body concentrations</td>
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<td>Exposure or test duration</td>
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<td>Ecologically important effects endpoints</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Decision rules for selecting protective tissue residue-effects information from individual publications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Residue-effects information</strong></td>
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<tr>
<td>Multiple effects endpoints for the same treatment</td>
</tr>
<tr>
<td>Multiple exposure scenarios</td>
</tr>
<tr>
<td>Multiple life stages</td>
</tr>
<tr>
<td>Multiple generations</td>
</tr>
<tr>
<td>Genders reported separately</td>
</tr>
<tr>
<td>Multiple LERs or NERs reported (i.e., temporal observations or replicates, and no mean reported)</td>
</tr>
</tbody>
</table>
in fish. The first method, referred to as the simple ranking approach, graphed the ranked NER and LER values for each contaminant on a log_{10}-scale. This provided a visual assessment of the range and distribution of the raw data. Plotting the log_{10} values facilitated data display by reducing skewness and making all bars visible on the graph. Initially, all life stages were combined for each contaminant. Subsequently, to reduce data variability, life stages were separated into two groups: Early life stages (ELS): egg, embryo, fry and juveniles and adults, collectively referred to as adults. The protective tissue threshold-effect concentration consistently was selected as the highest NER concentration below the lowest LER concentration.

The second method, referred to as the empirical percentile approach, calculated the fifth and 10th percentiles from the LER data alone. Percentile calculations are less sensitive to outlier values and have been used to develop water, sediment, and tissue-effect guidelines. For example, Klapow and Lewis [22] selected the 10th percentile of the median lethal concentration (LC50) data to estimate acutely toxic concentrations for developing water-quality guidelines in California, USA. The National Status and Trends Program used a 10th percentile of synoptic sediment chemistry and effects information to calculate sediment guidelines [17]. Meador et al. [23] calculated a 10th percentile concentration of the low-effect data to develop a protective polychlorinated biphenyl tissue concentration for salmonids. All these previous investigators focused on the effects data and did not consider the no-effects data in their methods. For our work, both a fifth and 10th percentile of the LER data were calculated using a standard spreadsheet percentile function (i.e., Excel®, Microsoft, Redmond, WA, USA), which uses linear interpolation between observed percentiles, treating the first and last ranked values as the 0th and 100th percentiles.

The third method calculated a tissue threshold-effect level (t-TEL) similar to that used by the Canadian Council of Ministers of the Environment and the state of Florida, USA for derivation of sediment guidelines [18,24]. By definition, the incidence of effects below the sediment TEL is predicted to be rare. The TEL approach is an expansion of the empirical percentile approach that incorporates the distribution of the combined no-effect and effect data into the final number to derive a concentration above which the no-effect concentrations predominate. The t-TEL, as adopted from the sediment guideline, is defined as

\[
t-TEL = \sqrt{LER-L \times NER-M}
\]

where t-TEL = threshold effect level as the geometric mean of LER-L and NER-M; LER-L = 15th percentile concentration in the effects data set; and NER-M = 50th percentile concentration in the no-effects data set.

The tissue residue dataset used to calculate a t-TEL was similar to the sediment dataset used by the Canadian Council of Ministers of the Environment and the state of Florida. Both datasets include published laboratory and field studies, and sublethal and lethal effects. Our approach differs in that tissue concentration data were taken from studies using only paired-effect and no-effect numbers, thereby ensuring that the threshold for adverse effect from each study was bounded.

In the fourth and most complex method, we estimated threshold effect concentrations using cumulative distribution functions (CDF). The CDF approach is used in species-sensitivity distributions and was used to derive national ambient water-quality criteria [16]. Species-sensitivity distributions are extrapolation models that fit a limited species dataset to a particular distribution with the goal of predicting effects to a larger set of species. Typical distributional forms assumed for this type of data include lognormal [25] and log-logistic [26], although nonparametric methods also have been used (e.g., [27]). For the species-sensitivity distribution approach to be valid, the dataset should consist of a randomly distributed collection of species representative of the community to which the guidelines will be applied.

For the tissue residue data, we fit a CDF for both the lognormal and log-logistic distributions. Sample parameter estimates were made using the least-squares methods employed in the literature (e.g., [19,28]). With the mean and standard deviation estimates calculated, the fifth percentile values, or hazardous concentration for 5% of the species (HC5), for the two distributions were found in standard statistical tables [29]. The 95% lower confidence limit of the fifth percentile also was computed using coefficients reported in the literature (normal – 25; logistic – 26). In this analysis, distribution fits were made for both the LER endpoints as well as the geometric means of the LER and NER endpoints for both chemicals. The geometric mean was used as an approximation of the threshold-effect concentration because some studies we reviewed reported large differences among dosing intervals and, thus, wide separation in corresponding LER and NER values.

Adequacy of the two hypothesized distributions (lognormal and log-logistic) was assessed using a delta-corrected one-sample Kolmogorov-Smirnov (K-S) test [30]. The appropriate critical values for this K-S test are based on an intrinsic hypothesis, i.e., that the population parameters were estimated from the data (Table Y from Rohlf and Sokal [31]).

If neither distribution was rejected by the K-S test, a method based on likelihood functions [32] was used to select from the two hypothesized distributions. The likelihood function for a particular distributional hypothesis computes the joint probability of obtaining the observed data under that hypothesized distribution, i.e., it presents the plausibility for a model based on the cumulative evidence from all data points. The relative likelihood, \(\pi(0)\), is computed as the individual model likelihood divided by the sum of the likelihoods for the two models. The largest relative likelihood was used to identify the most plausible of the two model choices for the data.

If a distributional hypothesis is rejected by the K-S test, then the parametric CDF approach should not be used for that distribution. Nonparametric alternatives to this CDF approach are based on bootstrapping when sample sizes are sufficient \((n \geq 20\) for \(HC_5\) estimates [27]). For discussion purposes, we report the CDF results for all datasets for both distributions.

Although the decision to use a fifth percentile, or \(HC_5\), is arbitrary, the number has become widely used [20]. We chose to use a \(HC_5\) to be consistent with the protective approaches used in the other three methods.

The same datasets were analyzed using all four methods. Replicate species data (i.e., data from different studies using the same species) were not combined or eliminated. The reasonableness of the estimated threshold-effect concentrations for the four methods was assessed by comparing them to both the geometric means of control organisms reported in the papers (Table 3) and to ambient tissue residue concentrations from fish captured in areas unaffected by point sources of contaminants [15]. Calculated protective tissue thresholds concentrations that fall at or below these control or ambient concentrations would be considered unreasonable thresholds.
However, because control and ambient residues could be lower than the no-effects tissue residues, they cannot be used to judge whether calculated protective levels are underprotective.

RESULTS

Literature review

Mercury. Using guidelines from Table 1, a total of 10 papers containing mercury residue-effect information for eight fish species were identified using guidelines from Table 1. One of the original 17 papers [41] was not included because tissue concentration units were reported inconsistently and personal communications with the authors failed to verify the correct units. Twenty paired NER/LER values and associated biological endpoints were obtained using the decision rules in Table 2. Eleven of the paired NER/LER values were for ELS and nine for adult fish (Table 3). Most laboratory exposures used technical-grade DDT or its active ingredient, \( p,p'\)-DDT. The approximate composition of technical-grade DDT is 77\% \( p,p'\)-DDT, 15\% \( o,o'\)-DDT, 4\% \( p,p'\)-DDE, 0.4\% DDD, and trace amounts of other compounds [42]. Most studies employed aqueous laboratory exposures with or without DDT-contaminated food. However, five papers evaluated effects of DDT in field-collected fish [43–47]. All but one [47] determined fry mortality from hatchery-spawned adults. Mortality was the biological endpoint measured most frequently in the DDT papers. Other endpoints included sublethal responses such as growth, behavior, and reproduction.

<table>
<thead>
<tr>
<th>Species by chemical and life stage</th>
<th>NER</th>
<th>LER</th>
<th>Control</th>
<th>Effect endpoint</th>
<th>Form and route of exposure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury, adult life stages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Walleye</td>
<td>0.06</td>
<td>0.25</td>
<td>0.06</td>
<td>Reproduction</td>
<td>MeHg, food</td>
<td>[66]</td>
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<tr>
<td>Striped mullet</td>
<td>0.1</td>
<td>0.3</td>
<td>&lt;0.1</td>
<td>Development</td>
<td>MeHg, aqueous</td>
<td>[67]</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>0.1</td>
<td>0.39</td>
<td>0.1</td>
<td>Reproduction</td>
<td>MeHg, food</td>
<td>[38]</td>
</tr>
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<td>Mummichog</td>
<td>0.21</td>
<td>0.44</td>
<td>0.08</td>
<td>Lethality</td>
<td>MeHg, food</td>
<td>[39]</td>
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<tr>
<td>Golden shiner</td>
<td>0.23</td>
<td>0.52</td>
<td>0.04</td>
<td>Behavior</td>
<td>MeHg, food</td>
<td>[65]</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>0.62</td>
<td>1.2</td>
<td>0.22</td>
<td>Growth</td>
<td>HgCl, aqueous</td>
<td>[36]</td>
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<td>0.079</td>
<td>0.86</td>
<td>0.079</td>
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<td>[40]</td>
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<td>Brook trout</td>
<td>2.7</td>
<td>5</td>
<td>0.1</td>
<td>Lethality</td>
<td>MeHg, aqueous</td>
<td>[37]</td>
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<tr>
<td>Mercury, early life stages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainbow trout (eggs)</td>
<td>0.02</td>
<td>0.07</td>
<td>0.02</td>
<td>Lethality</td>
<td>HgCl, aqueous</td>
<td>[35]</td>
</tr>
<tr>
<td>Rainbow trout (larvae)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td>Lethality</td>
<td>HgCl, aqueous + sediment</td>
<td>[35]</td>
</tr>
<tr>
<td>Grayling (fry)</td>
<td>0.06</td>
<td>0.27</td>
<td>0.06</td>
<td>Behavior</td>
<td>MeHg, aqueous</td>
<td>[68]</td>
</tr>
<tr>
<td>DDT, adult life stages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goldfish</td>
<td>0.06</td>
<td>1.65</td>
<td>0.06</td>
<td>Behavior</td>
<td>( p,p')-DDT, aqueous</td>
<td>[69]</td>
</tr>
<tr>
<td>Pinfish</td>
<td>0.067</td>
<td>0.55</td>
<td>0.067</td>
<td>Lethality</td>
<td>( p,p')-DDT, food</td>
<td>[70]</td>
</tr>
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<td>Lake trout</td>
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<td>0.29</td>
<td>0.19</td>
<td>Lethality</td>
<td>DDE, aqueous + food</td>
<td>[71]</td>
</tr>
<tr>
<td>Brook trout</td>
<td>0.61</td>
<td>11.2</td>
<td>0.61</td>
<td>Growth</td>
<td>Technical DDT, food</td>
<td>[72]</td>
</tr>
<tr>
<td>Chinook salmon</td>
<td>0.62</td>
<td>3.65</td>
<td>0.62</td>
<td>Lethality</td>
<td>Technical DDT, food</td>
<td>[73]</td>
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<tr>
<td>Cutthroat trout</td>
<td>0.8</td>
<td>1.1</td>
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<td>Lethality</td>
<td>( p,p')-DDT, aqueous</td>
<td>[56,57]</td>
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<td>Brook trout</td>
<td>2.8</td>
<td>7.6</td>
<td>0.6</td>
<td>Reproduction</td>
<td>Technical DDT, food</td>
<td>[74]</td>
</tr>
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<td>Fathead minnow</td>
<td>41.0</td>
<td>112.7</td>
<td>0.2</td>
<td>Lethality</td>
<td>Technical DDT, aqueous</td>
<td>[75,76]</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>10.4</td>
<td>33.8</td>
<td>ND</td>
<td>Lethality</td>
<td>Technical DDT, food</td>
<td>[73]</td>
</tr>
<tr>
<td>DDT, early life stages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic croaker</td>
<td>0.01a</td>
<td>0.07</td>
<td>NDb</td>
<td>Behavior</td>
<td>( p,p')-DDT, adult food</td>
<td>[48]</td>
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<tr>
<td>Rainbow trout</td>
<td>0.178</td>
<td>1.15</td>
<td>0.178</td>
<td>Lethality</td>
<td>DDT, adult food</td>
<td>[44]</td>
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<tr>
<td>Brook trout</td>
<td>0.21</td>
<td>0.89</td>
<td>0.21</td>
<td>Lethality</td>
<td>Technical DDT, food</td>
<td>[74]</td>
</tr>
<tr>
<td>Brook trout</td>
<td>0.21</td>
<td>11.92</td>
<td>0.21</td>
<td>Lethality</td>
<td>Technical DDT, food</td>
<td>[77]</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>0.3</td>
<td>1.27</td>
<td>0.2</td>
<td>Lethality</td>
<td>DDT, maternal transfer</td>
<td>[45]</td>
</tr>
<tr>
<td>Spotted seatrout</td>
<td>0.5</td>
<td>1.5</td>
<td>&lt;0.5</td>
<td>Lethality</td>
<td>DDT, maternal transfer</td>
<td>[47]</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>0.5</td>
<td>1.1</td>
<td>0.5</td>
<td>Behavior</td>
<td>( p,p')-DDT, maternal transfer</td>
<td>[46]</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>0.66</td>
<td>1.09</td>
<td>0.15</td>
<td>Lethality</td>
<td>Technical DDT, aqueous</td>
<td>[78]</td>
</tr>
<tr>
<td>Winter flounder</td>
<td>1.08</td>
<td>1.11</td>
<td>0.57</td>
<td>Lethality</td>
<td>Technical DDT, aqueous</td>
<td>[78]</td>
</tr>
<tr>
<td>Lake trout</td>
<td>2.67</td>
<td>2.93</td>
<td>NAc</td>
<td>Lethality</td>
<td>DDT, maternal transfer</td>
<td>[43]</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>6.8</td>
<td>24.0</td>
<td>0.4</td>
<td>Lethality</td>
<td>Technical DDT, aqueous</td>
<td>[75,76]</td>
</tr>
</tbody>
</table>

\( a \) Control = geometric mean of control replicates.
\( b \) Dry weight converted to wet weight concentrations using 80\% moisture.
\( c \) \( DDE = \) dichlorodiphenyldichloroethylene.
\( d \) Detection limit estimated from analytical method.
\( e \) Not detected, detection limit not provided.
\( f \) NA = not available.

DDT. A total of 17 papers containing DDT residue-effect information for 13 fish species were identified using guidelines from Table 1. One of the original 17 papers [41] was not included because tissue concentration units were reported inconsistently and personal communications with the authors failed to verify the correct units. Twenty paired NER/LER values and associated biological endpoints were obtained using the decision rules in Table 2. Eleven of the paired NER/LER values were for ELS and nine for adult fish (Table 3). Most laboratory exposures used technical-grade DDT or its active ingredient, \( p,p'\)-DDT. The approximate composition of technical-grade DDT is 77\% \( p,p'\)-DDT, 15\% \( o,o'\)-DDT, 4\% \( p,p'\)-DDE, 0.4\% DDD, and trace amounts of other compounds [42]. Most studies employed aqueous laboratory exposures with or without DDT-contaminated food. However, five papers evaluated effects of DDT in field-collected fish [43–47]. All but one [47] determined fry mortality from hatchery-spawned adults. Mortality was the biological endpoint measured most frequently in the DDT papers. Other endpoints included sublethal responses such as growth, behavior, and reproduction.
Data analyses

Method 1 (simple ranking approach): Mercury. Paired NER/LER values for all life stages in Table 3 were ranked from low to high (Fig. 1a). Most NER values are less than the LER values. Concentrations span about two orders of magnitude and all but three of the residues are less than 1 mg/kg. If one examines only adult and juvenile fish, all LER values are above a tissue residue concentration of 0.25 mg/kg (Fig. 1b). The highest adult mercury NER concentration below the lowest LER was 0.23 mg/kg. For the limited amount of ELS data (n = 3 studies), the lowest LER was 0.04 mg/kg and the highest NER below the lowest LER was 0.02 mg/kg (Fig. 1c).

Method 1 (simple ranking approach): DDT. Plotting the ranked paired NER/LER values for all life stages from Table 3 on a log_{10} scale also results in a gradual transition from low to high tissue concentrations (Fig. 2a). However, in contrast to the mercury residues, the NER and LER values are distributed more randomly. Tissue residues span nearly four orders of magnitude. As with mercury, the ranked NER/LER values were plotted separately for adults (Fig. 2b) and ELS (Fig. 2c). The threshold between adult LER and NER DDT values is less distinct than that observed for mercury. For example, six of the nine adult DDT NER values rank above the lowest LER (Fig. 2b). The lowest LER for adults was 0.29 mg/kg and the highest NER below the lowest LER was 0.19 mg/kg. The threshold between ELS NER and LER concentrations was more distinct (Fig. 2c). The lowest LER was 0.07 mg/kg. The highest NER below this LER was a nondetected result and the original paper did not report detection limits [48]. Results from the simple ranking approach are summarized in Table 4 along with results from the other three methods.

Method 2 (empirical percentile approach). The adult LER data yielded mercury tissue concentrations of 0.26 mg/kg and 0.28 mg/kg for the fifth and 10th percentiles, respectively (Table 4). Percentiles were not calculated for the ELS mercury results due to the paucity of data (n = 3 LERs). The limited mercury ELS dataset also precluded realistic evaluation of the
Table 4. Simple ranking, empirical percentile, tissue-threshold effects level (t-TEL), and cumulative distribution function (CDF) results for Hg and DDT in fish (mg/kg wet wt)

<table>
<thead>
<tr>
<th></th>
<th>Simple ranking</th>
<th>Empirical percentile</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Highest NER&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lowest LER&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hg Adult</td>
<td>8</td>
<td>0.23</td>
</tr>
<tr>
<td>Hg ELS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>DDT adult</td>
<td>9</td>
<td>0.19</td>
</tr>
<tr>
<td>DDT ELS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> NER (no-effect residue) = highest no-effect tissue concentration.<br><sup>b</sup> LER (low-effect residue) = lowest effect tissue residue concentration.<br><sup>c</sup> Range from two best-fitting models from Table 5.<br><sup>d</sup> ELS (early life stage) = egg, embryo, fry.<br><sup>e</sup> Insufficient data to do empirical percentile, t-TEL, or CDF approach.<br><sup>f</sup> ND = not detected, detection limit not provided.

t-TEL and CDF approaches. For DDT, the fifth and 10th percentiles were 0.39 mg/kg and 0.50 mg/kg, respectively, for adults and 0.48 mg/kg and 0.89 mg/kg, respectively, for ELS (Table 4).

Method 3 (tissue TEL approach). This approach, which uses both the NER and LER values, resulted in a mercury t-TEL of 0.21 mg/kg for adult fish (Table 4). As with the empirical percentile approach, a mercury t-TEL was not calculated for ELS. The DDT t-TELS for adult and ELS fish were 0.64 mg/kg and 0.70 mg/kg, respectively.

Method 4 (probabilistic CDF approach). The hypothesis that the distribution of data conformed to a lognormal distribution was rejected for the two DDT ELS datasets (α = 0.05, Table 5), and the hypothesis of a log-logistic distribution was rejected for the DDT ELS and adult mercury LER datasets. In order to illustrate the parametric CDF approach, the most plausible distribution was selected based on the relative likelihoods for the three geometric mean datasets. The HC<sub>5</sub> values determined by the two distributional assumptions differed by 0.5 to 1.5 orders of magnitude for the LER datasets. The HC<sub>5</sub> values from the lognormal distribution were higher, due to the smaller skewness inherent in the lognormal distribution. The relative likelihoods for the three geometric mean datasets did not indicate a strong probability for one distribution over the other. The π(θ) values for the geometric mean datasets ranged from 0.40 to 0.68 for the lognormal and 0.32 to 0.60 for the log-logistic (Table 5). When the relative likelihoods were similar for the two distributions, the resulting HC<sub>5</sub> values also were similar. Both early life-stage DDT LER distributions were rejected based on the K-S test (α = 0.05, Table 5).

Lower confidence bounds provide a measure of the uncertainty in the HC<sub>5</sub> estimates. For all mercury and DDT best-fit models, the lower confidence bounds were always 1 to 2 orders of magnitude smaller than their corresponding HC<sub>5</sub> values (Table 5). This indicates the considerable uncertainty present in these HC<sub>5</sub> estimates, due to the small sample size and variability of the acceptable data.

Comparison to control and ambient tissue residues

Mercury in control fish (0.09 mg/kg) was about two to three times lower than the adult threshold-effect concentrations calculated using three of the four methods: Simple ranking, per-

Table 5. Cumulative distribution function (CDF) results using log-normal and log-logistic models for mercury and DDT in fish (mg/kg wet wt)

<table>
<thead>
<tr>
<th>n</th>
<th>Endpoint</th>
<th>Log-normal</th>
<th></th>
<th></th>
<th>Log-logistic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K-S&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HC&lt;sub&gt;5&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>LCL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>π(θ)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>K-S&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hg Adult</td>
<td>LER&lt;sup&gt;e&lt;/sup&gt;</td>
<td>(0.138, 0.193)</td>
<td>0.11&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.9E–02</td>
<td>0.89</td>
<td>(0.189, 0.300)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>geomean&lt;sup&gt;e&lt;/sup&gt;</td>
<td>(0.189, 0.237)</td>
<td>0.048</td>
<td>7.2E–03</td>
<td>0.47</td>
<td>(0.130, 0.209)</td>
</tr>
<tr>
<td>DDT adult</td>
<td>LER</td>
<td>(0.051, 0.131)</td>
<td>0.085&lt;sup&gt;†&lt;/sup&gt;</td>
<td>3.2E–03</td>
<td>0.87</td>
<td>(0.112, 0.212)</td>
</tr>
<tr>
<td></td>
<td>geomean&lt;sup&gt;e&lt;/sup&gt;</td>
<td>(0.073, 0.170)</td>
<td>0.037&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.3E–03</td>
<td>0.68</td>
<td>(0.112, 0.212)</td>
</tr>
<tr>
<td>DDT ELS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>LER</td>
<td>(0.195, 0.261)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.7E–02</td>
<td>0.99</td>
<td>(0.274, 0.340)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>geomean&lt;sup&gt;e&lt;/sup&gt;</td>
<td>(0.182, 0.248)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.056</td>
<td>8.3E–03</td>
<td>0.40</td>
<td>(0.156, 0.223)</td>
</tr>
</tbody>
</table>

<sup>a</sup> K-S = test statistic for the delta-corrected one-sample Kolmogorov-Smirnov goodness of fit test, for delta = 0 and 1, respectively.<br><sup>b</sup> HC<sub>5</sub> = hazard concentration for estimated 5% of species, the 5th quantile for the specified distribution.<br><sup>c</sup> LCL = 95% lower confidence bound on the HC<sub>5</sub>, Tolerance limit (a lower confidence bound on a percentile).<br><sup>d</sup> π(θ) = relative likelihood for the particular distributional hypothesis, θ.<br><sup>e</sup> LER (low-effect residue) = lowest effect tissue residue concentration.<br><sup>†</sup> Best-fit distribution (highest relative likelihood) for each endpoint.<br><sup>‡</sup> Critical value exceeded (α = 0.05), significant deviation from the specified distribution.<br><sup>§</sup> Geomean = geometric mean of the individual paired no-effect-residue/LER values from each study.<br><sup>¶</sup> ELS (early life stage) = egg, embryo, fry.
Fig. 3. Geometric mean of tissue concentrations in control fish (horizontal line labeled Control) compared to protective tissue concentrations derived from simple ranking (R), empirical percentile (P5 = fifth percentile; P10 = 10th percentile), tissue threshold effect level (t-TEL), and the best-fit cumulative distribution function (CDF) for mercury in adult fish (a), DDT in adult fish (b), and DDT in early life-stage (ELS) fish (c). The DDT was not detected in ELS fish for the simple ranking approach (*).

Concentrations of mercury and DDT in fish collected from areas located away from known point sources of pollution is a second independent measure for judging the reasonableness of protective levels generated by the four methods. Currently, no accepted national fish residues exist for this type of comparison. However, the U.S. EPA conducted a national survey of chemical residues in fish that included 39 background stations located throughout the United States in areas relatively free of pollution sources [15]. These data can provide an indication of ambient concentrations in fish tissue in a field setting away from known sources of pollution. Approximately three to five adult fish of similar size and from the same species were collected and composited at each location. These fish represent a variety of bottom-feeding species. The geometric mean mercury in these fish was 0.11 mg/kg (n = 34). This value is comparable to the mercury in control fish (Table 1) and about half the concentration of threshold residues generated by three of the four methods: Simple ranking, empirical percentile, and t-TEL methods (Table 4). Because of biomagnification, ambient mercury residues in predatory fish likely are higher than in bottom-feeding fish.

For DDT, the U.S. EPA study [15] only reported DDE fish residues. The geometric mean for DDE at the U.S. EPA background locations was 0.02 mg/kg (n = 29). This value is over an order of magnitude below total DDT concentrations generated by the simple ranking, empirical percentile, and t-TEL methods for adult fish and about half the concentration predicted by the CDF approach (Table 4). The background DDE concentration also was an order of magnitude lower than the geometric mean of total DDT concentrations in the adult control fish (0.26 mg/kg).

Due to the paucity of information for mercury in ELS fish (n = 3 studies), the threshold-effect concentration (0.02 mg/kg) was calculated using only the simple ranking approach. This concentration approximates the geometric mean of the control organisms (0.03 mg/kg) from all three ELS studies. The protective residue of 0.02 mg/kg produced by the simple ranking approach was the mercury concentration in the control treatment from a single experiment [35]. This egg concentration is higher than concentrations observed for fish eggs from five species inhabiting the Great Lakes (range 0.004 mg/g to 0.011 µg/g wet weight [49] and at the high end of the range measured in walleye eggs from North America, 0.005 µg/g to 0.03 µg/g wet weight [50]).

DISCUSSION

Literature review

Attempts to derive protective tissue residues for fish continue to be hampered by a paucity of high quality, toxicological studies specifically designed to link residues and biological effects. We increased the size of the existing compilation by Jarvinen and Ankley [11] by including ecologically important behavioral test endpoints such as foraging behavior and predator avoidance. Although sometimes difficult to quantify, these behavioral effects can impact adversely and significantly survival and reproduction [51–53].

Deriving protective tissue residues also is hampered by a lack of consensus in the scientific community regarding the treatment and analysis of published residue-effect information. Using explicit decision rules and a consistent bias toward pro-
tive tissue concentrations helped reduce the inherent variability among experimental designs and endpoints. This bias, we feel, was validated independently by the a posteriori comparisons to control residues (Fig. 3) and ambient field tissue concentrations. The guidelines in Tables 1 and 2 were tailored to meet the needs of this particular collection of mercury and DDT papers. These guidelines likely have broad applicability, although additional or different decision rules may be appropriate for other contaminants.

We caution against the approach of using tissue residue information from only one study of a species that closely is related to the species of interest. This approach severely censors an already limited database and forces one to rely exclusively on the design, conditions, and endpoints of one or perhaps a few experiments. Taking paired NER and LER values from multiple studies has the advantage of including a variety of study designs and species to help ameliorate inter-experimental variability. This variability has been noted by other investigators who cited it as a major obstacle to establishing the relationship between tissue residues and biological effect [54]. Our approach of selecting paired NER/LER values has helped achieve our goal of identifying the threshold for adverse-effects area of the dose-response curve.

Methods comparison

Simple ranking approach. The first method we evaluated, the simple ranking approach, allows one easily to see the richness/paucity of the data, the range of concentrations, and the nature of the threshold between the NER and LER values. Potential outliers are easy to recognize. The simple ranking presentation also led us to separate life stages (ELS vs. adults) to provide a sharper threshold between NER and LER concentrations. Partitioning the life-stage data revealed only two adult mercury NER values interspersed among the LER data. The sharp threshold for the adult mercury data probably was influenced by the high quality of available studies: Closely spaced dosing concentrations, subtleth endpoints, generally low concentrations in controls, trace-metal–free protocols, and use of the same form of mercury. We recommend using the simple ranking approach as a first step for initial data screening and review when evaluating residue-effect literature.

The simple ranking approach revealed a much less distinct threshold response for the DDT data. That is, the NER and LER values overlapped across a wider concentration range compared to the pattern observed for mercury. This higher variability may have resulted from several features specific to the DDT literature. First, different DDT forms were used and measured in the experiments we reviewed. According to Lotufo et al. [9], the metabolites of DDT have differing toxicities and are better assessed by using a toxic unit approach. This approach was not possible in the present study. Second, because DDT is hydrophobic, variability in fish lipid content may have obscured residue-effect relationships. For this paper, DDT concentrations could not be lipid-normalized because most papers did not report lipid concentrations. The more distinct NER–LER threshold in ELS fish may be due to their less variable lipid content relative to older fish [55]. Because lipid-normalization potentially could reduce one source of variation, we recommend that lipids be analyzed in all future DDT experiments reporting tissue residues. A third reason the DDT threshold response was less distinct than mercury’s is the fact that DDT studies were conducted in the 1960s and 1970s when analytical methods were less precise and less accurate. The older analytical techniques were less able to distinguish the various forms of DDT from other chlorinated hydrocarbons and, therefore, may have biased upward reported DDT concentrations. Finally, elevated DDT concentrations in control fish (Table 3) may have obscured the exposure-response relationship. The DDT residues in many of the control treatments from these older papers exceed ambient field concentrations by an order of magnitude. The DDT contamination of fish food was a problem reported by several investigators (e.g., [44,56,57]). The possible influence of DDT-contaminated food is unknown, but test organisms chronically exposed to this food source may have developed resistance to DDT, resulting in a higher and more variable threshold for adverse effects. These issues with the older data add uncertainty to the development of a protective threshold for DDT.

The ELS DDT dataset contained one extremely low LER concentration (0.07 mg/kg) reported by Faulk et al. [48]. We feel this data point is qualitatively different from the other LERs because fish were exposed to the $o,p'$ form of DDT (a minor component of technical-grade DDT), a subtleth behavioral endpoint was examined (the majority of other DDT studies tracked fish survival), and this was a recent study relative to the other DDT literature. Another recent study using a sensitive sublethal endpoint (immunosuppression) reported an effect to salmon fry at 0.02 mg/kg $o,p'$-DDE in whole-body [56]. Additional studies using sensitive endpoints are needed to confirm these low DDT tissue-effect concentrations.

Using the simple ranking approach and selecting the highest NER below the lowest LER value as the threshold concentration provides a protective number with no effects reported below it. A main limitation with this approach arises when extreme values are present, such as the Faulk et al. [48] paper discussed above. Consequently, for this ELS DDT dataset, the threshold effect concentration selected using the simple ranking approach gives a below detection limit concentration and appears inconsistent with the majority of the data (Table 4).

Empirical percentile approach. The second method we evaluated, the empirical percentile approach, has more flexibility than the simple ranking approach because the user may select the desired level of protection. We calculated the fifth and 10th percentiles of the LER data based on approaches used by other investigators and to ensure that the effect concentration was protective. An important advantage of the empirical percentile approach is the reduced influence of extreme values in the dataset [22]. For example, the fifth percentile of the ELS DDT dataset is 0.48 mg/kg even with the inclusion of the extremely low LER concentration (0.07 mg/kg of [48]). The empirical percentile approach is a nonparametric approach and, thus, requires no assumptions about the distributional form of the data. This approach also can be used on relatively small datasets, although estimates of extreme percentile values ($\leq$5th or $\geq$95th) have large confidence bounds even for substantial datasets. Although calculating a percentile appears to be a simple and straightforward approach, users of this method should be aware that a number of different models exist for calculating percentiles. Some percentile models strictly treat the sample as the population, i.e., the lowest-observed value is treated as the 0th percentile and the highest-observed value as the 100th percentile (e.g., Excel uses this model). Other percentile models assign the percent rank as rank/($n + 1$); extrapolation beyond the percent ranks of the observed data are not possible without specification of absolute upper and lower bounds. Using this latter model for the empirical
percentile approach, a hazardous concentration for the fifth percentile of affected species cannot be estimated when sample sizes are less than 19 [59]. With this latter model, our smallest data set, with eight LER concentrations for mercury, has its first ranked data point (0.25 ppm) at the 11th percentile of the dataset.

Another characteristic of the empirical percentile approach is that only the lower concentrations drive the final number when taking a low percentile of the dataset. In other words, the addition of higher effect concentrations to the dataset will not impact the calculated low percentile effect concentration. Because our goal was deriving a protective threshold number, this attribute of the empirical percentile approach confers stability to the derived effect concentration.

Tissue TEL approach. The third approach, adapted from the sediment guidelines literature [24], resulted in protective levels that seemed to represent results generated by both the simple ranking approach as well as the empirical percentile approach (Table 4). The t-TEls for adult mercury and DDT were, respectively, slightly lower and higher than outputs from the simple ranking and the empirical percentile approaches, respectively (Table 4). The t-TEL for ELS mercury was intermediate to the fifth and 10th percentiles of the empirical percentile approach. As discussed above, the CDF approach generated protective levels well below the other three methods and at or below control residues. Although the simple ranking and empirical percentile approaches use only the separate NER or LER datasets, the t-TEL approach incorporates both NER and LER distributions. Because it incorporates both data distributions, the t-TEL is the only percentile-based method that represents all the available data. Using the percentile designated by the sediment guidelines (50th percentile of no-effects and 15th percentile of effects) appears reasonable with the datasets used here, but should be re-examined as additional residue-effects papers are published. For this review, we were unable to meet the minimum 20 data point requirement specified in the sediment TEL guidelines [24].

Cumulative distribution function. The probabilistic CDF approach, with its goal of extrapolating to the whole community or population from a limited dataset, is a very appealing method. However, to make this extrapolation accurate and reasonable, specific data requirements need to be met. Primarily, the data should be a random sample from the general population. If the sample produces biased estimates of the population mean and variance, for example due to dominance by either sensitive endpoints or insensitive species, then the thresholds resulting from this approach may be unrealistically low or high.

The data used in this study came from a wide variety of exposure scenarios, contaminant forms, and endpoints that inflated the variability of these data beyond simple interspecific differences. The term cumulative distribution function was adopted to avoid association with species-sensitivity distributions. Species-sensitivity distributions typically describe the variation among a set of species from laboratory toxicity tests where results from consistent laboratory test procedures and effect endpoints are expected to yield differences that are based on intrinsic species differences, not experimental design or endpoint differences. Existing tissue residue datasets for mercury and DDT do not meet these requirements.

The CDF results were evaluated both for goodness-of-fit of the lognormal and log-logistic models and for the reasonableness of the predicted HC₅ values. Neither model demonstrated a consistently better fit based on the K-S test and relative likelihoods. The K-S test is driven by one data point (the maximum deviation from the hypothesized distribution) and so is particularly sensitive to outliers. In general, the goodness-of-fit test results should be complemented by graphical investigations (e.g., quantile-quantile plots, or empirical cumulative distribution functions, with the hypothesized curves overlain). Other parameter estimation techniques besides the least-squares methods used here also are available and may provide distribution parameters that are a better fit for the data. The likelihood approach used to compare among models can be extended to any number of distributional hypotheses including alternative parameter estimates.

The threshold-effect concentrations predicted by the CDF approach were lower than threshold-effect concentrations predicted by the three other methods, synoptic control tissue residues (Table 3), and ambient mercury (but not DDT) tissue residues from field collections. Therefore, for these datasets, the CDF approach is not viewed as particularly useful for estimating threshold-effect concentrations in whole bodies of fish. The results of the CDF approach also confirm the difficulty in identifying an underlying distribution for small datasets, and agree with conclusions voiced by other investigators regarding the limitations of this approach [28,60,61]. If other investigators take a CDF approach, the models should be evaluated for fit as well as for the reasonableness of the predicted HC₅.

Recommendations

Based on results presented in Table 4 and our recommendation to consider the t-TEL approach a superior method, the following tissue residues are deemed protective of fish. For mercury, we recommend 0.2 mg/kg whole body as protective for juvenile and adult fish. This number largely is based on sublethal endpoints and additional studies likely will not substantially alter this number. As reported above, the geometric mean of mercury in adult bottom-feeding fish from background locations throughout the United States is 0.11 mg/kg, approximately one-half our recommended protective level. Due to the paucity of information, we cannot derive a protective level for mercury in ELS fish using the t-TEL approach. Only the simple ranking method could be used on the ELS mercury data providing a protective concentration of 0.02 mg/kg.

In recommending a whole-body approach for mercury tissue residue calculations, Niimi and Kissoon [62] concluded that lethal body burdens of mercury fell into the 10 to 20 mg/kg range. Based on the literature available to them in 1994, they speculated that sublethal impacts would fall into the 1- to 5-mg/kg range. With a number of recent high-quality publications, our analyses indicate that sublethal effects to mercury can occur at concentrations well below the 1 to 5 mg/kg predicted by Niimi and Kissoon [62] for adult fish. Weiner and Spry [63] concluded that sublethal effects to embryonic and larval stages could occur at 1 to 10% of the adult concentrations. Effects of mercury to early life stages of fish, including eggs, have been measured at extremely low concentrations [35]. In their study of reproductive effects of methylmercury and polychlorinated biphenyls, Matta et al. [39] observed reduced fertilization success in Fundulus heteroclitus at egg concentrations below the detection limit of 0.02 pg/g. Additional ELS fish studies using low detection limits are needed to validate the protective concentration of 0.02 mg/kg we derived for mercury in ELS fish.
For DDT, we recommend protective levels of 0.6 mg/kg in whole-body adult fish and 0.7 mg/kg for ELS fish. As reported above, the geometric mean of DDE in adult fish from background locations throughout the United States is 0.02 mg/kg, an order of magnitude lower than our recommended protective level. These recommended concentrations may not be fully protective because the results were derived from older studies that emphasized lethality rather than the potentially more sensitive sublethal endpoints. Use of a safety factor with these provisional DDT residues may be appropriate. Additional studies, such as those of Faulk et al. [48], should be conducted to determine whether our provisional recommendations for DDT residues truly are protective and reasonable. The influence of lipid normalization on the provisional DDT residues of 0.6 to 0.7 mg/kg also should be evaluated. In addition, studies that evaluate the toxicity of the different metabolites are needed. Therefore, these DDT tissue residue-effect numbers should be considered provisional and used carefully.

For many chemicals, insufficient data have been published to derive protective tissue residue concentrations for fish. The recent publication of tissue residue guidelines for selenium for the protection of aquatic life is in contrast to information available for many other bioaccumulative chemicals [64]. We strongly encourage investigators to conduct studies designed specifically to produce technically sound residue-effect information. Tissue residue analyses also should consider inclusion of endpoints in addition to those used by Jarvisen and Ankley [11]. Adverse effects on fish behavior, especially during sensitive life stages, could have important ecological implications. Maternal transfer (i.e., dose to developing eggs) is an important, yet often ignored exposure pathway in fish and should be evaluated. We recommend experimental designs such as ones used by Hammerschmidt et al. [38], Drevnick and Sandheinrich [40], Faulk et al. [48], and Webber and Haines [65] as providing valuable guidelines, endpoints, and results (e.g., at least 3 dosing concentrations, multiple sensitive endpoints evaluated, exposure through diet, rigorous data analyses).

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