Species Assessments under the Endangered Species Act and The Federal Insecticide, Fungicide and Rodenticide Act

Using Best Available Scientific Methodology to Address the Needs of both Acts

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Endangered Species Issue Management Team

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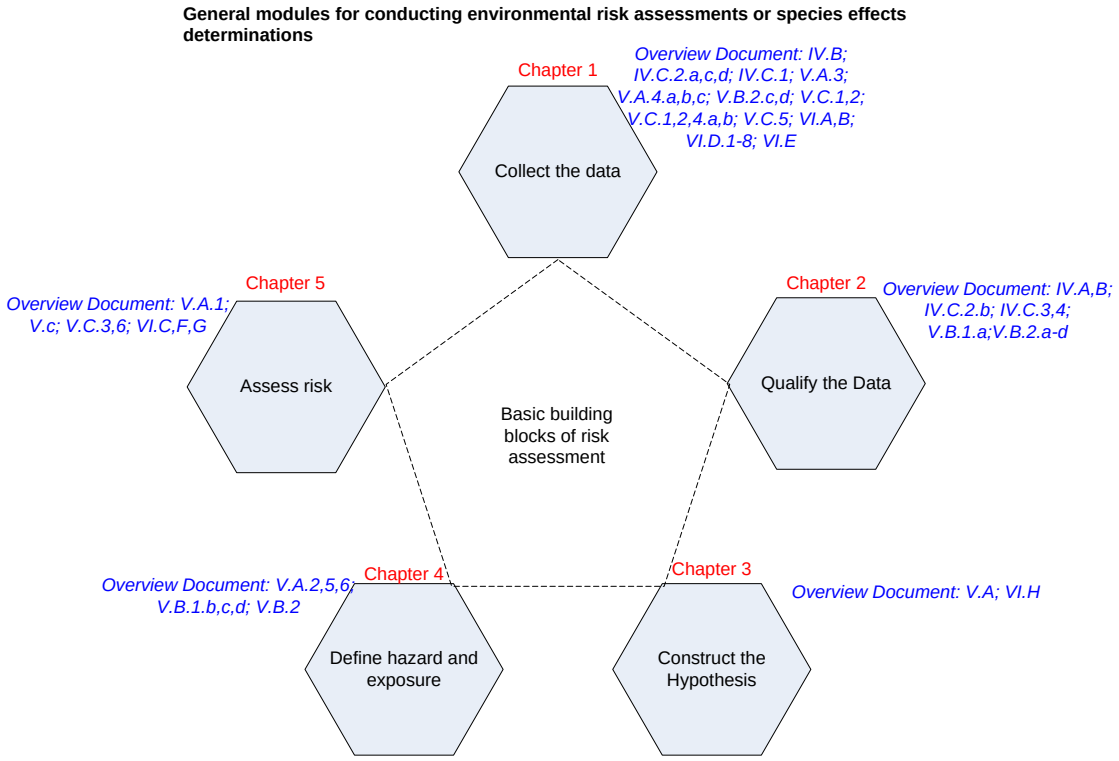
EXECUTIVE SUMMARY

The complex processes of ecological risk assessment of pesticides for registration actions conducted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and of species effects determinations conducted under the Endangered Species Act (ESA) are essentially equivalent and compatible from a risk assessment perspective, even though certain definitions or endpoints vary in the underlying regulations. Although there are differences in statutory language and purpose between FIFRA and the ESA, for both laws the objective of the risk assessment is to determine whether, and to what extent, there may be a risk of adverse effects to protected species. Thus there is no difference in scientific principles applied to assessing risk under either statute. A risk assessment conducted under FIFRA can meet the requirements of a species effects determination under ESA and retain the overall degree of protection for listed species required by the ESA and the Counterpart regulations (50 C.F.R. § 402.04; 51 Fed. Reg. at 19937), using the methodology developed by consensus between EPA and the Services (i.e., National Marine Fisheries Service (NOAA-Fisheries) and Fish and Wildlife Service (FWS)). This document (1) describes the synergisms between assessments conducted under the two regulatory statutes, (2) proposes the efficiencies of a synchronous, standardized, coordinated approach to risk assessment to address the requirements of both regulations simultaneously in one document, (3) identifies the best available science and the uncertainties within it, and (4) describes viable methods for managing uncertainties.

When the FIFRA risk assessment process, as described in EPA's "Overview Document[1]," is used to address the scientific aspects of consultation needs under ESA, experience and use of best available data and scientific methods result in a robust analysis. Documentation of that analytical process is generally described in the Overview Document, but the scientific limitations and challenges presented in some areas, and procedures for dealing with them, are not completely clear. This document provides some suggestions for developing the details in the Overview Document into a process that will add transparency and which can be used to conduct species and risk assessments to meet both FIFRA and ESA needs. Presented here is a review of methods and underlying policies that ultimately shape the full FIFRA risk assessment (or, for ESA, the effects determination) and resulting risk management process. Supporting rationale presented herein are intended to supplement or enhance the Overview Document, contributing to the understanding of a robust scientific approach for analysis, thus providing the basis for sound policy and risk management decisions.

[1] USEPA OPPTS OPP (2004). *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency: Endangered and Threatened Species Effects Determinations.* January 23, 2004

There are certainly minimum standards for adequate risk assessment methodologies, but there are multiple avenues available to the risk assessment process. It is important to move along these avenues in a methodical way, starting with a base of solid data of low uncertainty (structured and validated laboratory and in some cases field data) and moving to the inclusion of data of less certainty where it qualifies for use. Documentation of why certain endpoints are not or cannot be evaluated is also important. This document examines areas like this as well. Specifically, the first chapter examines the treatment of sublethal effects, mixtures and toxicity endpoints (including levels of concern and risk quotients) in the risk assessment process, and defines the overarching structure of the risk assessment process. Unlike the classic "Risk=Hazard + Exposure" diagram, described here are the activities that support the process, as follows:



As mentioned above, the first module of the assessment process is to gather the data necessary to support the assessment. While it is recognized that the formal process in risk assessment called *Problem Formulation* (a means of describing how the assessment will progress) and its underlying hypothesis will describe the data that should be sought, under FIFRA a standardized testing scheme has been developed to support the best methodologies available in risk assessment. This is a constantly evolving process and test parameters and the nature of tests required, along with the risk assessment methods applied to them, change over time. In the FIFRA environment, testing guidelines which have evolved over time use the best available science to apply a suite of studies to a given situation. This suite of "guideline studies" becomes the data addressing the registration requirements for a given product, supported by additional data from the open literature when it is reliable and relevant.

The current state of the science does not often support drawing conclusions regarding the significance of "sublethal" effects – i.e., there is little valid data that supports a hypothesis that non-lethal behavioral effects observed in the laboratory, for example, will be reasonably expected to manifest themselves in the field in a way that impacts survival, growth or reproduction. It is rare that any such cause-and-effect relationship can be demonstrated and quantified. Thus, in the vast majority of cases, there is neither data indicating that sublethal effects would pose risk to listed species, nor a scientifically accepted process for quantifying any such risk.

Some have argued that the assessment procedures in use do not adequately address the potential for sublethal effects. However, the current endangered species risk assessment approach addresses potential sublethal effects through measurement or observation endpoints developed during testing conducted under acute and chronic exposure conditions. Sublethal endpoints that can be used to assess both direct and indirect effects on listed species and their critical habitats are evaluated via registrant-submitted studies conducted according to USEPA guidelines for test methodology and quality. In EPA's Overview Document (USEPA, 2004a), examples of sublethal endpoints are given for aquatic organisms, birds and mammals. EPA acknowledges that additional sublethal endpoints are potentially available through an evaluation of the open literature, when such endpoints arise from peer-reviewed, validated testing methods. Indirect effects and critical habitat assessments inherently utilize both lethal and sublethal endpoints. The taxonomic groups addressed by the evaluation equate to the varying trophic levels in the environment, as well as to potential habitat requirements for a species.

It is important to recognize that the conservatism of the endangered species risk assessment is protective of the species under assessment because it utilizes multiple "worst case" assumptions that are highly unlikely to occur in the natural environment. Additionally, the Services extensively evaluated this approach in its review of EPA's FIFRA risk assessment process (Services, 2004) and reported that they endorse the appropriateness of the methods and EPA's approach to interpreting sublethal and indirect effects in the risk assessment process. In this review, Williams and Hogarth (Services, 2004) note that, with respect to endangered species risk assessment, the techniques EPA uses are also useful for the assessment of effects on designated critical habitat.

The assessment of the risks associated with so-called "mixtures" is another area of discussion within this document. As EPA acknowledges, the current state of available data on the co-occurrence of multiple chemicals is sparse and meager, and is not sufficient to allow EPA to draw valid science-based conclusions regarding potential exposures. Whatever data are available and relevant should be considered, and if mixtures are specifically stated on the pesticide label, EPA will review the label and application rates. If the additive rates are more than the rate of either product used alone, this will be considered in an assessment, and registration will not be granted until EPA's concern for application rates and/or the allowed mixtures are addressed.

With respect to hypotheses concerning the possibility of synergistic, antagonistic or additive effects of pesticides, EPA has determined that there is currently no valid and accepted method for determining any such risks; nor is there data showing that there is reason to believe that such effects would make any significant difference in the risks that EPA calculates. Certainly, there are presently no validated *in vitro* test and short-term *in vivo* test methods that are specifically developed to assess the potential for interactions in complex mixtures. Consequently, professional judgment must be applied to the risk assessment process to determine whether or not environmental combinations of pesticides are of concern. With few exceptions, the scientific literature regarding toxicity of mixtures, particularly pesticide mixtures, has shown that with an adequate dosing level, when toxicological interactions occur from exposure to a mixture of pesticides, the interaction typically is additive. Best available data, reviewed later in this document, suggest that synergism, antagonism and potentiation do not occur at expected environmental concentrations of pesticides. The data for both humans and other species suggest that the vast majority of the time, when chemicals are present in a mixture at sufficient levels to cause toxicity, the interaction is additive or less.

Many of the advances in risk assessment science are in the area of improved exposure modeling. OPP EFED utilizes validated exposure models applied in a sequential fashion to the risk assessment process. There are many models available, but not all meet the requirements for relevancy and peer-reviewed utility when applied in the environmental risk assessment process. Terrestrial models, conservative in that they are biased toward overestimating exposure, are just now emerging and are of limited but some usefulness in the environmental risk assessment process. There is a scarcity of models for assessing exposure in urban settings or other non-crop settings. Validated and reliable models have been developed for predicting exposure, and thus risk, to aquatic systems and organisms. However, this is the state of best available science/data. The risk assessment process anticipates that it generally is not possible to model certain circumstances, such as for inert compounds or mixtures beyond a Tier I screening level due to a lack of proper characterization data.

One important factor in developing exposure estimates is estimating uncertainty due to model inputs. This is particularly important, since it is known that standard EPA models typically provide estimated concentrations that greatly exceed measured levels, sometimes by several orders of magnitude.

Chapter 2 moves to the next module of risk assessment, that of qualifying the available information for use in risk assessment. In the risk assessment process, EPA utilizes primarily the studies conducted in compliance with Agency guidelines and submitted by the registrant as required under FIFRA. These studies are high quality and designed to be the principal data set for conducting ecological risk assessments (including endangered species analysis). Guideline studies are conducted under strict Good Laboratory Practice (GLP, 40 CFR 160) standards including: validated testing methods; well characterized test substance; detailed testing protocols; and a high level of documentation and independent quality assurance; and other features such as highly controlled environments, standardized strains of animals for tests, etc. These studies are subject to the most

rigorous criteria in their generation and acceptance, and are considered validated and reproducible.

The risk assessor supplements the FIFRA data by relying on peer reviewed public literature when it is adequate for or meaningful to the risk assessment being conducted. While the peer-review process confers some credence to the information contained in a journal article, it does not necessarily bestow equal quality of such data to data developed under FIFRA and GLP guidelines, nor does it indicate that data from a peer-reviewed article is of high enough quality or contextually relevant for regulatory decision-making. Additionally, FIFRA procedures allow the consideration of the benefits of pesticide use, but ESA procedures do not. A large portion of the target organisms that prompt a pesticide application in rangeland, right-of-way, forestry and aquatic settings, however, are invasive species. Invasive species displace native species, especially endangered species because they are those most vulnerable to stressors. This creates an entirely different risk assessment paradigm than simple pest control within a crop habitat. When considered together, invasions by exotic plants, animals, and pathogens are regarded by biologists as one of the major threats to biological diversity worldwide. Consequently, in denying the use of an herbicide for the control of invasive species, the agency taking such steps may actually be promoting what the ESA describes as a "take." This means that even for endangered species matters, in making a decision on stressors the benefits of pesticides must be considered. In the formal process in risk assessment called *Problem Formulation* (a means of describing how the assessment will progress), and/or in the discussion of stressors, one question that needs to be considered is whether or not the pesticide under evaluation will be used in such a way as to relieve the effect of other stressors on non-target species.

Chapter 3 describes the formulation of the risk hypothesis, the third module of the assessment process as described by this document. Here, additional elements enter into the assessment process such as the consideration of whether (1) additives to the end use formulation affect toxicity or exposure; (2) degradates are of concern and need to be considered in the risk equation; (3) the taxa for which data are available represent the species of concern; (4) typical exposure routes are adequate to define exposure in the expected circumstances; and (5) simulation of exposure using traditional approaches is adequate. As scientific methods for developing exposure profiles advance and are validated, new approaches to simulating exposure will be adopted as the best available method for defining an exposure profile. Currently, the uncertainty of the contribution made by pathways not specifically included in the exposure profile is in part, if not wholly, addressed by the method used for estimates of dietary exposure.

The risk assessor must determine if there are other components of the pesticide that require evaluation, and therefore the risk assessor will take steps to characterize any possible alterations in exposure to the environment which could be caused by inert and other ingredients (adjuvants, degradates and impurities) found in or added to crop protection end use formulations. The process of considering whether special attention needs to be given to the inert ingredients or impurities in an end use formulation, as well as the need to address degradates of the active ingredient that result upon storage or

environmental degradation, relies on data generated by the FIFRA testing process and the experience of the risk assessor.

Chapter 4 discusses how the hazard and exposure evaluations of the risk assessment process are built. Regulatory agencies have recognized the complexity of dealing with environmental variables and that the conservatism built into the risk assessment process is the only currently available method to deal with the various uncertainties such as species spatial and temporal status, environmental contaminants in existing habitats, and the effect of multiple stressors on an organism or population. In the point-specific assessment, such as one conducted for a building site, these factors are more identifiable, but on a national scale assessment, such as that required for a registration action, it is impossible to describe all the conditions in all of the areas where a pesticide may be used. It then becomes important to address those elements that are most significant in the assessment of risk and that can be addressed at the national level (for example, lack of proximity or extirpation of a species in a given county), moving to more specific geographic and risk analysis when necessary to draw a sound scientific conclusion.

It is important to examine some of the components of ESA that are relevant to a point-specific evaluation, but are out of context in a national-level assessment. For example, the Services are charged with describing the "environmental baseline" for a given species and action. National level "baselines" however, simply do not exist. On a national scale, the elements selected for review of the environmental baseline by sheer geography and number would be subject to selective and incomplete description and would not provide the context required for a discussion of the baseline adequate to allow a determination as to whether a pesticide use produces significant and measurable direct or indirect effects when compared to the stressors that could occur in any situation, anywhere in the nation. To a great extent, the FIFRA Endangered Species Task Force (FESTF) Information Management System serves to define a baseline on a national scale, by identifying where there is an intersection between a species and use, and by providing, over time, retrievable information on local circumstances or relationships between the species and potential use site.

Additionally, there is no reliable method for predicting the level and type of contaminants that *might* occur in any given circumstance (ESA's reference to "contaminant load"). Contaminant load is in fact a more detailed evaluation of the elements of a baseline, and thus less likely to be quantifiable even on a local basis. The ESA only requires that existing data be examined, and thus the best available approach to the issue of contaminant load is addressed by other aspects of the risk assessment process, such as expert knowledge on the behavior of the chemical in the environment.

Developing a Problem Formulation and defining an Action Area, per ESA needs, are both under further scientific development. The science of risk assessment is an evolving one, and the approach to problem formulation and action area definition is currently receiving a good deal of attention. As definition is assigned to these elements of the risk assessment process, risk assessors will adaptively manage their approaches to meet best available documentation and methodology. A second aspect of ESA that is somewhat

manageable on a local level but unquantifiable at the national level is that of dealing with cumulative effects.

Chapter 5 deals with how the risk and the uncertainty of the risk determination are evaluated. Very often the line between the scientific risk assessments and the risk management decisions based upon them becomes blurred. This circumstance is magnified when the basic premise for the risk assessments are not fully explained or when the risk assessor does not fully address the needs of the risk manager. A failure to communicate combined with professional differences of opinion exaggerates what actually may be similar opinions. The differences between scientists can be reduced by careful attention to a clearly defined risk hypothesis, an understanding and explanation of protection goals and a description of what constitutes "jeopardy." Each of these three areas is very broad and each is lacking in quantitative definition, which would help reduce the uncertainty in the risk assessment process. While EPA's FIFRA risk management goals are driven by the statue's "no unacceptable adverse effect," EPA enters an entirely separate process, with additional safety factors and at a higher tier, for addressing endangered species risk. The added safety factors and more rigorous investigation of species- or site-specific conditions account for the ESA statute's stricter protection goals. EPA then characterizes the risk in making its risk management decisions.

Chapter 6 concludes the document by noting that in conducting risk assessments, EPA utilizes the best available methods and data to characterize the risk presented by pesticides to endangered species. These methods meet the statutory demands of both FIFRA and ESA with respect to the protection of endangered species.

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Chapter One: State of the Scientific Process as Applied To Risk and Species Assessments

1.1 Introduction

The complex processes of ecological risk assessment of pesticides for registration actions conducted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and of species effects determinations conducted under the Endangered Species Act (ESA) are essentially equivalent and compatible, even though certain definitions or endpoints vary in the underlying regulations. Legal and regulatory language differences do not equate to a difference in scientific principles. A risk assessment conducted under FIFRA meets the requirements of a species effects determination under ESA and retains the overall degree of protection afforded listed species. This document (1) describes the synergisms between assessments conducted under the two regulatory statutes, (2) proposes the efficiencies of a synchronous, standardized, coordinated approach to risk assessment to address the requirements of both regulations simultaneously in one document, (3) identifies the best available science and the uncertainties within it, and (4) describes viable methods for managing uncertainties.

FIFRA was first enacted in 1945 and thus was one of the first "environmental regulations" in effect. Over the years of its maturation, improvements in technology and scientific technique, as well as an improved understanding of our environment, have brought a reliable slate of validated study methods and environmental risk assessment methodologies. Results of environmental and toxicology testing are applied in the systematic risk assessment process with an intentional bias toward conservatism, resulting in risk management decisions that are protective within a given margin of uncertainty. The risk assessment process for FIFRA evaluations has evolved based on multiple guidelines for data development, emerging science, experience with the interpretation of test results, and advances in risk assessment science. FIFRA allows the Agency to demand data submittal from registrants prior to and at any time during or after the issuance of a registration. Post-registration data call-ins address areas of concern that might arise as science evolves. Therefore, unlike a contaminated site evaluation, or a point-specific land use evaluation, the FIFRA risk assessment process begins in a data-rich state, based on studies with endpoints that are directly relevant to the intended uses of the pesticide and has a mechanism built in it to keep the underlying data set the best available by constantly considering the best science and methodologies available.

Under the FIFRA process, the *use pattern* of a product under evaluation is known, and is factored into the interpretation of hazard and exposure. As EPA notes (USEPA, 2006b):

> *In developing human health and ecological risk assessments, EPA's risk estimates include:*
>
> - *Current label use rates and application practices;*
> - *Typical use rates and practices (usually lower than the maximum use rates on product labels); and*

- *Proposed use rates and practices that are being considered as part of the risk management/mitigation decision-making process, including mitigation options proposed by stakeholders as well as EPA risk managers.*

The main purpose of this document is to clarify the FIFRA risk assessment processes and to describe how these processes also meet the needs of other statues that intersect with FIFRA, primarily the Endangered Species Act. Examined here are the state of the science and the best methodologies that can now be applied to risk assessment, recognizing that as new science or methodologies are validated, the risk assessment process will be adapted to embrace them. Throughout this document are a series of explanations and flowcharts that correspond to the general modules of the evaluation process shown in Figure 1.1.

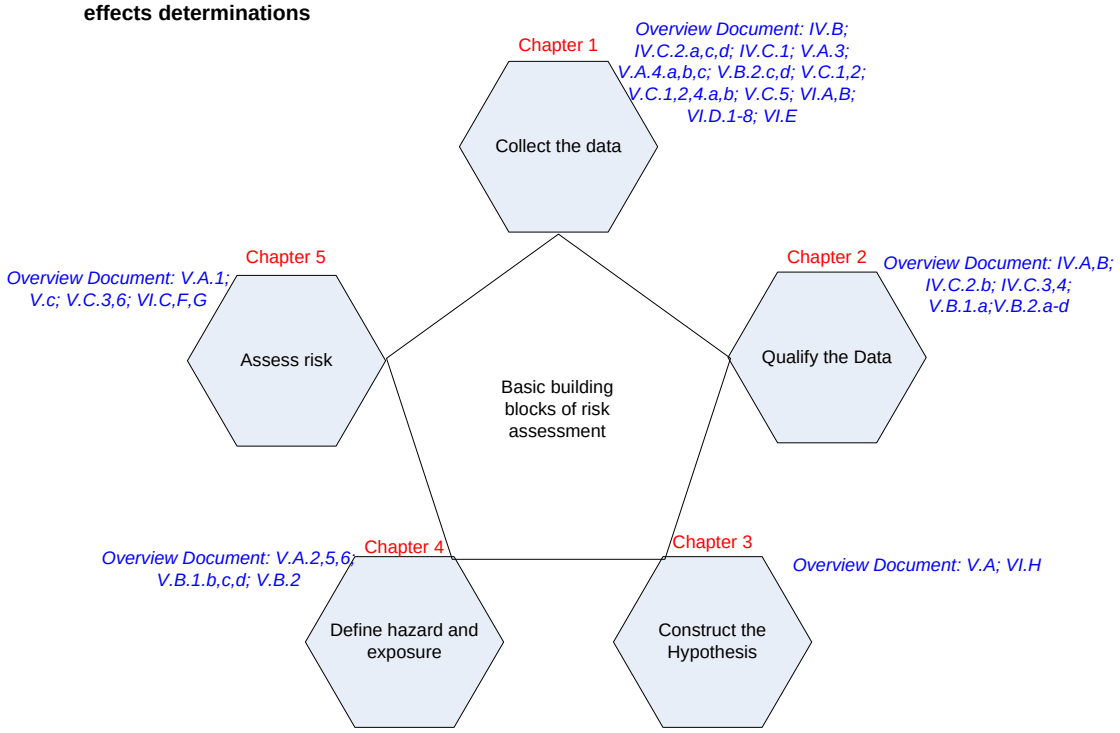
As Steve Williams and William Hogarth (Services) note in their letter to Suzan Hazen (EPA) (Services, 2004) responding to EPA's Overview Document (USEPA, 2004) and observed differences in ESA and FIFRA approaches (as described in a Services memo dated March 7, 2003),

> *When evaluating OPP's approach, the Services were mindful that there is no single correct approach to evaluating ecological risks. Organizations often use different, yet analytically defensible methodologies that are capable of producing sound, scientifically based effects determinations.*

There are certainly minimum standards for adequate risk assessment methodologies, but there are multiple avenues available to the risk assessment process. It is important to move along these avenues in a methodical way, starting with a base of solid data of low uncertainty (structured and validated laboratory and in some cases field data). Course exposure estimates are then applied through modeling and extrapolation of exposure and environmental behavior and distribution (factors and data that carry high uncertainty). The exposure assessment follows a tiered process that can be adapted for each assessment, allowing the assessor to develop a logical and efficient approach. The tiered process has been in use and is developing over time. This document seeks to formalize that tiered process with respect to the status of scientific methods (Chapter 1); how complex endpoints are addressed using state-of-the-science (Chapter 2); what techniques are used to extrapolate data across varying pesticide formulations or taxonomic classes of organisms (Chapter 3); the parameters of "best available data" (Chapter 4); and testing hypothesis and dealing with uncertainties (Chapter 5).

Specifically, this first chapter examines the treatment of sublethal effects, mixtures and toxicity endpoints (including levels of concern and risk quotients) in the risk assessment process.

Figure 1.1. General modules for conducting environmental risk assessments or species effects determinations



1.2 Defining Best Available Data and Methods

As mentioned above, the first module of the assessment process is to gather the data necessary to support the assessment. While it is recognized that the formal process in risk assessment called *Problem Formulation* (a means of describing how the assessment will progress) and its underlying hypothesis will describe the data that should be sought, under FIFRA a standardized testing scheme has been developed to support the best methodologies available in risk assessment. This is a constantly evolving process and test parameters and the nature of tests required, along with the risk assessment methods applied to them, change over time. Studies conducted by a registrant must follow strict methodology documented in standard and complete protocols and follow "EPA Guidelines for Pesticide Testing." These guidelines are not suggestions about how to conduct a test and the endpoints to be measured; they are very specific instructions for study development, conduct and documentation which must be followed for a study to qualify as fully addressing the needs of an endpoint or "data requirement." Each study must also follow codified Good Laboratory Practice Standards (40 CFR 160), which are ensured through not only a self-auditing process but also an EPA external auditing process. When registrant-conducted studies or studies from the public literature are disregarded in the assessment process, it is usually due to improper, inaccurate, invalidated or out-of-context reported endpoints. The logic of selecting studies is portrayed by the "Pyramid of Data Quality" (McGaughey, 2005) as shown in Figure 1.2

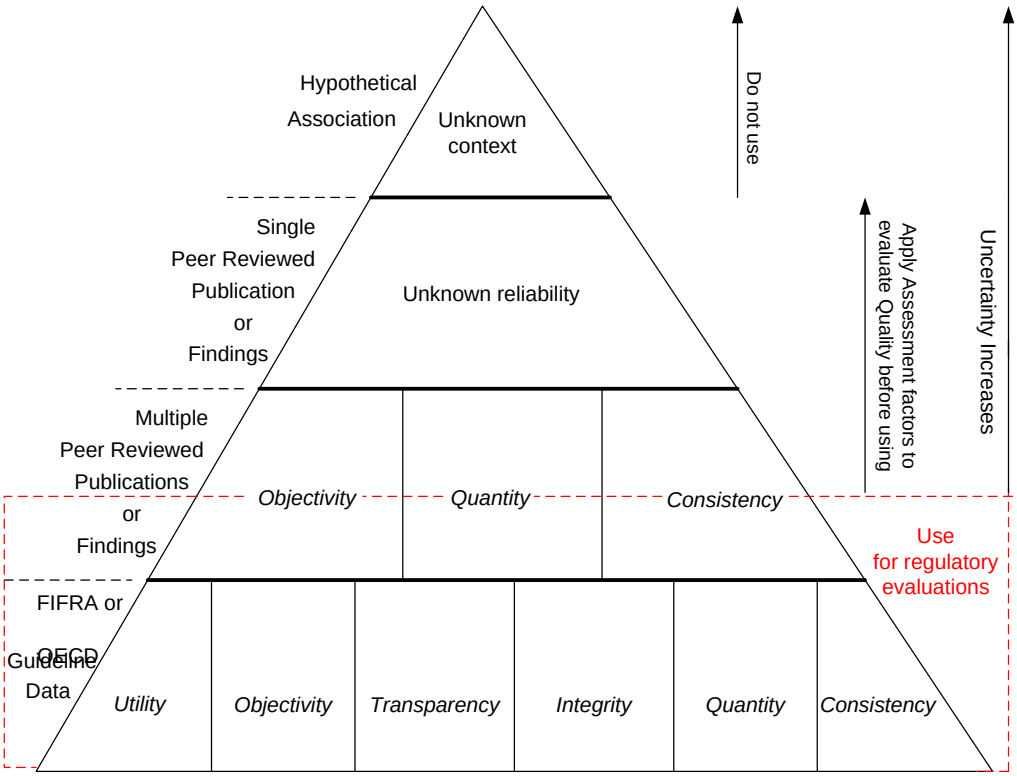
The approach illustrated in this diagram expands upon the basic and more limited quality criteria described by the Services in their draft approach to literature searching (Services, undated).

In the FIFRA environment, testing guidelines which have evolved over time use the best available science to apply a suite of studies to a given situation. This suite of "guideline studies" becomes the data addressing the registration requirements for a given product, supported by additional data from the open literature when it is reliable and relevant. There are testing standards and conditions that must be met for a given study or publication to be relevant and reliable. The collection of studies ultimately required and relied upon in the risk assessment will be based on the properties of the product as well as the results of sequential tests, where end points in the first levels of testing "trigger" the next set of testing. In addition to the registrant submitted studies, reviews of the open literature provide an evaluation and determination on whether test methods and endpoints meet the quality standard, and are validated and relevant to pesticide use. The parameters of data quality are discussed further in Chapter 4.

Similarly, risk assessment methodologies follow a structured process described by various EPA internal and external "instruction manuals." One of the first efforts to harmonize the approach to environmental risk assessment and document the procedure was a basic framework for ecological risk assessment (USEPA, 1992). From the methodology in this document arose specific guidelines to replace it (USEPA, 1998), which were codified in a Federal Register Notice in May of that year (63 FR 26846). To further instruct risk assessors in the assessment and communication of risk, EPA published a Science Policy Handbook on Risk Characterization (USEPA Office of Science Policy, 2000). This document stresses the need for transparency, clarity, consistency and reasonableness in the risk characterization process. The science of risk assessment continues to evolve and new methods likely will be developed and refined as scientific methods and the regulatory review process permit. The current process, which proceeds in a tiered manner as uncertainty is addressed, will continue to be refined as new methods are validated and used for a more focused look at hazard and exposure.

EPA's Office of Pesticide Programs (OPP) evaluates available public literature in endangered species assessments. This evaluation includes consideration of both lethal and sublethal effects. If there is sufficient and reliable information characterizing the assumed sublethal effect and functional endpoints, and the study meets data quality standards and has relevance to the assessment, then the sublethal endpoint can be used in risk assessment. EPA has previously described how publicly available literature is captured and evaluated in the ECOTOX database (USEPA, 2004a). Data that pass the ECOTOX screen are evaluated and may be incorporated qualitatively or quantitatively into the endangered species risk assessment (see also Chapter 4).

Figure 1.2: Proposed Data Quality Pyramid for Risk Assessment Processes and Risk Management Decisions (McGaughey, 2005)



"Best available" loses reliability as it sheds its components of quality

FIFRA Guideline Data: Has its *utility* defined by FIFRA testing guidelines; has its*consistency* defined by EPA review; has its *quantity* defined by FIFRA data requirements; has its *objectivity, transparency* and *integrity* assured by GLP requirements.

Repeated Peer Reviewed Publications or Findings*: Has consistency defined by replication; demonstrates quantity based on the statistical design of the studies; has its objectivity from peer review; has utility to the extent it supports risk assessment; but loses its integrity and transparency because methods are not documented to the degree GLP's require.*

Single Peer Reviewed Publications or Findings: Has limited *objectivity* depending on the level of peer review, but has its *utility* defined by one circumstance and may not have been designed for purposes of risk assessment; and loses its *transparency b*ecause methods are not documented to the degree GLP's require, loses its *quantity* by its isolation and is of unknown *consistency*.

Hypothetical Association: Has virtually no *utility;* loses its *objectivity* to subjective speculation; has no *transparency* in methodological scientific application; is not supported by any *quantity* of data; and has no measure for *consistency.*

1.3 Sublethal Effects

The current endangered species risk assessment approach addresses potential sublethal effects through measurement or observation endpoints developed during testing conducted under acute and chronic exposure conditions. Sublethal effects are effects other than mortality that could alter the dynamics of a species population. A classic example of a sublethal effect is eggshell thinning in raptors (Ratcliffe, 1967; Lincer, 1975). Reported effects on olfaction, predator avoidance, and homing behavior of salmonids (Scholz et al., 2000; Moore and Waring, 1996) are other examples. (It should

be noted that the impact of these effects on populations of raptors and salmonids, respectively, is still subject to debate; see for example Green, 1998, Powell and Palm, 2007a,b).

Sublethal endpoints that can be used to assess both direct and indirect effects on listed species and their critical habitats are measured in registrant-submitted studies conducted according to FIFRA guidelines. In EPA's Overview Document (USEPA, 2004a), examples of sublethal endpoints measured in guideline studies are given for aquatic organisms, birds and mammals. Sublethal endpoints observed and recorded in registrant-submitted acute and chronic guideline studies are described below.

- Acute studies
    - Fish acute studies report dose-related abnormal behavior such as erratic swimming, loss of reflex, increased excitability, lethargy and changes in appearance.
    - Avian acute studies include estimates of food consumption, descriptions of intoxication, observations of abnormal behavior, and records of the time of onset, duration and severity of these symptoms as well as the number of test organisms affected at each dose/concentration level.
    - Invertebrate acute studies report abnormal behavior or appearance. For studies on certain organisms, other sublethal endpoints are included which are relevant for those particular organisms. For example, a common endpoint for assessing potential effects on mollusks is oyster shell growth. The alteration of this endpoint, shell growth, would indicate a sublethal effect.
    - Mammalian studies are generated primarily as surrogates for the human health risk assessment, but these studies are also directly relevant to risk assessment on other mammalian species. Typically, rats, mice and rabbits are used in acute testing and the endpoints measured include observations on behavior, physiology and activity.

- Chronic studies
    - Fish early life-stage studies, in addition to recording cumulative mortality and length and weight, consider time of development (time to start of hatching and end of hatching, number of larvae hatching each day), number of deformed larvae, as well as abnormal behavior (e.g. hyperventilation, uncoordinated swimming, atypical quiescence, and atypical feeding behavior). Full life-cycle studies, if triggered by the results of early life-stage studies, are occasionally conducted and further explore potential sublethal chronic effects. Doses selected for these studies are intentionally set at levels to elicit sublethal chronic effects *but not mortality.*
    - Avian chronic studies consider mortality, growth, and several reproductive endpoints, including egg production and the fertility and fecundity of the

parent organism. Other sublethal effects include eggshell thickness, food consumption, behavioral changes, and gross pathology.

- Invertebrate life-cycle studies are designed to detect effects on growth and reproduction, while abnormal behavior and appearance are also reported. The significance of behavioral effects in the absence of effects on reproduction is not straightforward, however.
- Mammalian studies give a wealth of information on potential sublethal effects. Separate studies are conducted on oncogenic, reproductive, teratogenic, behavioral and neurotoxic effects.

In addition to the studies and endpoints described above, test guidelines for evaluating effects on endocrine production and function in mammals, fish, and aquatic invertebrates are currently being validated.

As acknowledged in EPA's Overview Document, additional information on sublethal endpoints is sometimes available in the open literature. If information arises from peer-reviewed, validated testing methods, and if a scientifically sound relationship can be established between the measured sublethal effect and assessment endpoints such as survival or reproductive capacity, the sublethal effect can be used quantitatively or qualitatively in the risk assessment. Use of a sublethal endpoint in the assessment will be based on the quality of the study, the relevance of the sublethal effect to survival and reproduction, and relevance to potential environmental exposure conditions.

The Services extensively evaluated EPA's approach in their review of EPA's FIFRA risk assessment process (Services, 2004):

> *OPP's risk assessment approach addresses potential sublethal effects through acute and chronic exposure conditions. OPP evaluates multiple sublethal endpoints for aquatic organisms, birds and mammals. For example, sublethal endpoints for aquatic species include embryo hatch rate, time to hatch, growth, exposed egg production, second generation hatch rate, and second generation growth. For screening risk assessments, OPP selects the most sensitive toxicity endpoints for establishing RQs (e.g., for aquatic species, the chronic RQ is based on the dose level at which none of these sublethal toxicity endpoints is effected, otherwise referred to as the NOEC), that are then compared to LOCs for chronic risk.*
>
> *The Services have deemed appropriate the existing sublethal endpoints that are included by OPP in its risk assessment process, and the manner in which they are used for purposes of assessing potential sublethal effects. While recognizing that these endpoints may establish the best available data for OPP's use, the Services also note that future consideration will be given to the development of additional sublethal endpoints. OPP has the option of including additional sublethal data in its risk assessment, if sufficient and reliable information establish a scientifically sound relationship between the proposed sublethal effect and the survival or reproductive capacity of an organism. The Services and EPA intend to hold future*

discussions about including additional sublethal endpoints in OPP's risk assessment process, subsequent to external peer review by the FIFRA SAP.

These findings endorse the appropriateness of EPA's approach to interpreting sublethal effects in the risk assessment process.

1.4 Indirect Effects and Effects on Designated Critical Habitat

In addition to assessing direct effects of pesticides on listed species, the current process assesses indirect effects on listed species and effects on designated Critical Habitat. Indirect effects may occur if the pesticide reduces the listed species' food supply or otherwise modifies the species' habitat. Indirect effects and critical habitat assessments use lethal and sublethal toxicity endpoints for taxa other than the listed species. As described above, toxicity data for primary producers (algae, aquatic vascular plants, and non-target terrestrial plants), primary consumers (herbivores), and secondary consumers (predators) are required in the pesticide registration process. There are some gaps that, from a practical standpoint, cannot be filled, such as testing top predators like eagles or grizzly bears. In examining the potential indirect effects on a listed species or effects on critical habitat, all of these endpoints can be used. In the screening level assessment, these analyses are generic in nature. However, once a potential risk is identified and the assessment moves beyond the screening level, the relative importance of the endpoints may vary with the species of concern and its biological and habitat requirements.

A simple example of an indirect effect is a significant reduction in the abundance of aquatic invertebrates upon which a listed fish species is dependent for food. If toxicity data indicate that the pesticide may affect the survival, growth, or reproduction of aquatic invertebrates, then the potential exists for indirect effects on fish that require aquatic invertebrates in their diet. Similar indirect effects could occur to herbivorous species due to reduction in plant growth (as indicated by toxicity data for plants), or to predators dependent upon a particular prey species (as indicated by toxicity data for the prey taxa). Many plant species are dependent upon insects for pollination, and pesticide effects on the insect pollinators may have indirect effects on the dependent plants. Conversely, some listed insect species are closely associated with particular plant species (for example, the Karner blue butterfly is dependent upon the wild lupine), and direct effects of pesticides on the required plants may cause indirect effects on the dependent insects. These and other potential indirect effects are assessed using the same toxicity endpoints (including sublethal endpoints) that are used to assess direct effects.

Williams and Hogarth (Services, 2004) describe the indirect effect assessment process in more detail:

During interagency discussions examining this risk assessment process, OPP and the Services discussed methods to ensure OPP's process uses appropriate available information to identify potential indirect effects during the screening level assessment. Through these discussions, the Services and OPP identified ways that existing LOCs established by OPP will inform their consideration of indirect effects on listed species.

- *If exposure levels do not trigger established taxonomic LOCs for any listed species, OPP can conclude that pesticide use will not cause indirect effects to listed species that may be dependent (as food, habitat, etc.) upon that taxonomic class of species.*
- *If exposure levels trigger established taxonomic LOCs for non-listed species, OPP will conduct a more thorough review of possible indirect effects. Exceeding this substantially lower threshold elevates concerns that non-listed species may be directly affected, which in turn raises concerns that listed species dependent upon such non-listed species may indirectly be affected as well. Under this scenario, OPP will proceed to more closely evaluate the nature, likelihood and magnitude of any indirect effects. This evaluation will review whether any listed species may be dependent upon a species within the class whose tests triggered the non-endangered species LOC, will review the nature of the dependent relationship, and will review the likelihood and magnitude of the potential indirect effects on the listed species due to the direct effects on the non-listed species, using the best scientific and commercial data available.*
- *If exposure levels trigger established taxonomic LOCs for listed species, but do not exceed established LOCs for non-listed species, OPP will exercise its professional judgment in determining the level of additional inquiry necessary to evaluate the potential for indirect effects. In exercising such judgment, OPP will in part document the actual probability of an effect (using data from its direct effects analysis), the listed species within the action area, and the nature and importance of any interrelationships between listed species and the non-listed species upon which they depend. If in the exercise of its professional judgment OPP determines that pesticide use may pose indirect effects to listed species, it will proceed to more closely evaluate the nature, likelihood and magnitude of any indirect effects using the best scientific and commercial data available.*

In assessing critical habitat, principal constituent elements (PCEs) that have been identified for the species provide the basis for this assessment. Principal constituent elements are those attributes of the habitat that are critical to the survival, stabilization and recovery of a listed species For each listed species not passing the screening level assessment with a “no effect” status, the Overview Document notes that an analysis of the PCE’s and all available endpoints is performed to determine their relevance for species-specific assessment. However, it is entirely possible that a listed species could pass the screening level assessment with a “no effect” status and still be vulnerable to indirect effects or effects on PCE’s. Therefore, current OPP assessments (e.g. those completed on the California Red-legged frog) assess indirect effects and PCE’s even when there is “no effect” on the listed species. This additional consideration applies below, as well, in the assessment of designated critical habitat.

Williams and Hogarth note that, with respect to endangered species risk assessment, the techniques EPA uses for indirect effects are also useful for the assessment of effects on designated critical habitat:

> *Through its risk assessment process, OPP considers the effects of pesticide use on designated critical habitat. During interagency discussions, OPP and the Services reviewed how OPP can use the available information to identify effects to critical habitat during the screening level assessment. If initial screening level toxicological tests indicate that the listed species LOC would not be exceeded, then no further analysis would be required, as OPP could reliably determine that no effect on critical habitat would be caused without further specific knowledge about the critical habitat. However, if a listed species LOC is exceeded, OPP would proceed to review the locations and elements of any designated critical habitat, and the potential effects of pesticide use on critical habitat in the following manner:*
>
> - *OPP will first determine whether any portion of the action area has been designated as critical habitat for any listed species. Through an internal database, OPP will identify all counties in which the pesticide at issue likely will be used, based upon known crop production within that county. OPP and the Services will work on a collaborative basis to identify any relevant counties that also include designated critical habitat.*
> - *Where the pesticide at issue will be used within critical habitat, OPP will determine whether pesticide use "may affect" critical habitat. To do so, OPP will examine relevant biological information concerning the habitat, including most specifically the critical habitat designation and the identified PCEs. OPP will then use available toxicity data to determine whether the pesticide "may affect" any PCE. For those PCEs that are an organism or can be characterized based on a functional relationship to an organism, this review will include a review of acute and chronic test data, to determine whether the pesticide may affect the class of species by which the PCE would be represented. If the toxicity analysis indicates that the listed species LOC would be exceeded, then OPP would determine that the pesticide may affect the PCE, and therefore may affect the critical habitat. OPP could also determine that the pesticide may affect critical habitat if toxicity data related to exposure indicated a non-biological PCE (e.g., water temperature as influenced by vegetation cover) would be altered due to the exposure to the pesticide.*
> - *If pesticide use "may affect" critical habitat, OPP will then determine whether pesticide use is likely to adversely affect critical habitat. This review will require a more thorough examination of the scope and magnitude of the effects on the relevant PCE, and will require additional review of biological data about the habitat, how it is used by the listed species, and the features that caused the affected portion to be designated a PCE. This review will be similar in analysis and relevant information to the assessment of indirect effects on the listed species, due to the nature of the dependent relationship of a listed species on its designated critical habitat. However, OPP will examine*

effects on critical habitat distinct from its analysis of effects on listed species, to ensure that pesticide use is not likely to result in destruction or adverse modification of critical habitat. OPP's assessment will be based upon the best scientific and commercial data available, which may include information from the critical habitat designation, listing notice, recovery plan, status reviews, and other relevant information, and include a review of the open literature consistent with the process identified in our earlier discussion of best scientific and commercial data available.

1.5 Protective Nature of the Conservative Risk Assessment Process

It is important to recognize that the conservative nature of the endangered species risk assessment is adequately protective of the species under assessment because it provides a combination of "worst case" assumptions that are extremely rare occurrences in the natural environment. For example, estimates of dietary exposure to birds and mammals assume a diet of short grass, which has the highest predicted residue concentration, despite the fact that no small birds or mammals could survive on a diet of short grass. Likewise, aquatic exposure is estimated using the assumption of a water body receiving runoff from a watershed that consists entirely of treated crop. Additionally, the assessment process uses the lowest reported toxicity value from acute studies on various species within diverse taxonomic categories, which assumes that all listed species within each category are at least as sensitive as the most sensitive species tested. Assumptions such as these, along with level of concern (LOC) criteria that represent an additional safety factor, build several layers of protection into the assessment process.

In its assessments, OPP uses the risk quotient (RQ) method to characterize risk. The RQ is a comparative number calculated by dividing the worst-case estimated environmental concentration (see Section 1.7) by the most sensitive effect value for a given taxonomic category. The RQs are compared to the LOC criteria. The LOCs are used "to indicate when a pesticide use as directed on the label has the potential to cause adverse effects on non-target organisms" (USEPA, 2004a). The risk presumption categories and corresponding LOC values which apply additional safety factors are illustrated in Table 1.1.

Table 1.1. Ecological Risk Presumptions, Risk Quotients and Levels of Concern (USEPA, 1998a)

Risk Presumption	Risk Quotient	Level of Concern
Aquatic Animals		
Acute High Risk	EEC[1]/LC_{50}	0.5
Acute Restricted Use	EEC/LC_{50}	0.1
Acute Endangered Species	EEC/LC_{50}	0.05
Chronic Risk	EEC/NOEC	1
Terrestrial Animals		
Acute High Risk	EEC/LC_{50} or LD_{50}/sqft[(2)] or LD_{50}/day[(3)]	0.5
Acute Restricted Use	EEC/LC_{50} or LD_{50}/sqft[(2)] or LD_{50}/day[(3)]	0.2
Acute Endangered Species	EEC/LC_{50} or LD_{50}/sqft[(2)] or LD_{50}/day[(3)]	0.1
Chronic Risk	EEC/NOEC	1
Aquatic and Terrestrial Plants		
Risk to Non-Endangered Species	EEC/EC_{50} (aquatic) or EEC/EC_{25} (terrestrial)	1
Risk to Endangered Species	EEC/NOEC or EC_{05}	1

[1]Estimated Environmental Concentration in water or avian/mammalian food items
[2](mg/ft^2)/(LD_{50} x wt of animal)
[3](mg of toxicant consumed/day)/(LD_{50} x wt of animal)

Williams and Hogarth (Services, 2004) describe the application of the LOC and its protective nature as follows:

> *LOCs are conservative RQs, which if not exceeded would generally lead EFED to conclude that pesticide usage has no effect on non-target species. The acute LOC for listed aquatic animals is an RQ of .05, which, depending on the dose response values observed in toxicity testing, translates into a risk of mortality ranging from one in 200, to less than one in 10^{16}. For a pesticide with an average slope of 4.5, the estimated risk is around one in 417,000,000. For listed terrestrial wildlife EFED's acute LOC is an RQ of 0.1, which translates into a risk of mortality ranging from one in 50 to less than one in 10^{16}. For a pesticide with an average slope of 4.5, the estimated risk is around one in 300,000. Acute LOCs for listed species are a fraction of those established for non-listed species. The chronic LOC for listed animals is an RQ of 1, which represents an exposure equivalent to a level producing no observed adverse effects. The listed plant LOC is an RQ of 1, which represents an exposure equivalent to either a level producing very limited effects (EC05) or no observed adverse effects depending upon the availability of effects data.*

Many factors embedded in the pesticide registration process give confidence that the ultimate risk assessment is adequately protective: it is supported by a relatively rich data base; there is a relatively long history of experience with the test systems and assessment approach; and there is a long track record of experience with it for hundreds of active ingredients. Moreover, because companies must report FIFRA 6(a)(2) events (incidents), it will be readily apparent if the system fails to identify a problem.

1.6 Toxicity and Risk Assessment of Pesticide Mixtures

The toxicological response to two or more chemicals present in a mixture has been classified as being expressed in one of the following four categories:

- Additive (the toxic effects are equal to the sum of the toxicity of the individual compounds);
- Synergistic (the toxic effects are greater than the sum of the toxicity of the individual compounds);
- Antagonism (the toxic effects are less than the sum of the toxicity of the individual compounds); or
- Potentiation (one compound has minimal toxicity, but combination with another compound renders it markedly more toxic).

EPA does not regulate based on mixtures. The requirements of FIFRA are directed to the evaluation of an individual pesticide product. EPA notes in its Overview Document (USEPA, 2004a) that:

> *The Agency does not routinely include in its screening level assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of product formulations of active ingredients, each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site.*

Toxicological Aspects of Evaluating Mixtures

The principles of toxicology applicable to mammalian species, such as humans and other vertebrates, are similarly applicable to endangered invertebrates and other ecological species, and thus to the risk assessment process as a whole. A review of the scientific literature regarding mixtures (Carpy et. al., 2000) evaluated data collected over a 14-year period from 1985-1998. While this focused on human exposure to chemical mixtures, the special emphasis on mixtures of pesticides at low doses (levels likely to occur in human diet and environment) is relevant to the levels evaluated in environmental exposure assessment. The study found:

> *Data have demonstrated that exposure to a combination of compounds does not cause effects stronger than the ones of their most active component, provided components are present at low concentration levels, like acceptable daily intake (ADI) or reference dose (RfD) levels, well below their respective no-observed-adverse-effect levels (NOAELs). Although it has been demonstrated that a combination of compounds with the same target organ and the same or very similar mechanisms of action may cause additive or synergistic effects, the chance of such effects will most likely diminish with decreasing exposure levels to such combinations. Synergism and antagonism may both occur at the same time at different organs or targets in the same organism. However, and despite some exceptions, it has*

> *been demonstrated that interaction between components is not a common event at low levels of human exposure such as those that may occur through pesticides residues in food or drinking water. The introduction of a special safety factor as a standard for mixtures in addition to those normally used for deriving ADIs, RfDs, or minimal risk levels is not supported by the data. It can be concluded from our review that, as a general rule, exposure to mixtures of pesticides at low doses of the individual constituents does not represent a potential source of concern to human health.*

Additional support for this finding is provided by Cassee and Groten (1998) and McCarty and Bogert (2006). McCarty and Bogert state:

> *Essentially, it appears that when organic chemicals are combined in concentrations below their individual thresholds for observable toxicity, they contribute additively toward the narcotic toxicity of the mixture proportional to their molar ratios in the mixture. This holds regardless of the type or mechanism of observable toxicity produced by the mixture components when administered individually in higher concentrations.*

The general approach for evaluating the potential adverse effects of pesticides in non-target species was established over four decades ago by Lehman, Fitzhugh and Nelson at the Food and Drug Administration, which was then responsible for regulating pesticides in the US. During the subsequent two decades preceding the creation of the EPA and the transfer of the major responsibility for regulating pesticides from FDA to EPA, several additions to the toxicity testing requirements were made to evaluate the hazard of pesticides. The discovery of organophosphate (OP) insecticide potentiation by Frawley at the FDA (Frawley, 1957) led to a requirement for testing of all new OP insecticides (as well as some other classes of pesticides) for such synergistic effects, and a leghorn chicken test was also required for all new organophosphate insecticides to detect any delayed neuromuscular effects of these agents. Significant developments have occurred in the areas of neurotoxicology, behavioral toxicology, and in immunotoxicology and have stimulated changes in EPA testing requirements when the endpoints and methodology were scientifically clear and validated.

OPP has often been challenged by the arguments surrounding the possibility of synergistic, antagonistic or additive effects of pesticides. As noted above, this is not a new concern, nor one that has gone unaddressed. Throughout the 1960's, after Frawley's discovery, it was common practice to conduct pesticide potentiation studies in rats on mixtures of chemicals whose structure or activity would suggest a possibility of increased toxic effects when an organism was exposed to the two pesticides at the same time. Many studies were conducted on organophosphates and other products. For example, seven studies are in the submission record for trichlorfon (EPA OPP, 1999) on various mixtures of organophosphates, including one study combining trichlorfon (an organophosphate insecticide) and dibrom (a brominated soil fumigant). After the conduct and submission of hundreds of studies, the requirement for pesticide potentiation studies was abandoned

because these studies provided no new information that was helpful to the risk assessment process.

Currently, there is no single methodological approach recommended to test the toxicity of environmental combinations of pesticides. Additionally, there are presently no validated *in vitro* test and short-term *in vivo* test methods that are specifically developed to assess the potential for interactions in complex mixtures. The safety factors built into the application of hazard data to the determination of a "level of concern" and a very conservative exposure modeling process are used to protect non-target organisms from any potential effects of environmental combinations of chemicals should they occur at toxicologically significant levels in the organisms' environment.

Carpy et al. (2000) discussed the state of the science of risk assessment for mixtures:

> *Despite a large body of knowledge in the field of risk assessment methodologies for exposure to chemical and pesticide mixtures, there is no single methodological approach in "combination toxicology" and health risk assessment of chemical mixtures, and therefore professional judgment is still required. Generally, the dose or response additivity approach that may be applied to evaluate potential risk for chemical mixtures in human toxicology overestimates the risk of a combination of chemicals.*

McCarty and Borgert (2006) reviewed the theory, policy and regulatory practices regarding the toxicity of chemical mixtures, and stated:

> *An analysis of current mixture theory, policy, and practice was conducted by examining standard reference texts, regulatory guidance documents, and journal articles. Although this literature contains useful theoretical concepts, clear definitions of most terminology, and well developed protocols for study design and statistical analysis, no general theoretical basis for the mechanisms and interactions of mixture toxicity could be discerned. There is also a poor understanding of the relationship between exposure-based and internal received dose metrics. This confounds data interpretation and limits reliable determinations of the nature and extent of additivity. The absence of any generally accepted classification scheme for either modes/mechanisms of toxic action or of mechanisms of toxicity interactions is problematic as it produces a cycle in which research and policy are interdependent and mutually limiting.*

With few exceptions, the scientific literature regarding toxicity of mixtures, particularly pesticide mixtures, has shown that with an adequate dosing level, when toxicological interactions occur from exposure to a mixture of pesticides, the interaction typically is additive. Best available data suggest that synergism, antagonism and potentiation do not occur at expected environmental concentrations of pesticides. In considering this issue

with respect to endangered species assessment, Williams and Hogarth (Services, 2004) note:

> *Pesticide mixtures, as discussed by the Services and EPA, fall into three separate categories: mixtures created by components added by the user, including surfactants or other additives; separate pesticide products used in combination with one another by the user; and mixtures occurring within the environment as a result of independent application of pesticide products and their interaction with environmental substances. At the present time, OPP's screening level assessment does not consider effects to non-target species caused by such mixtures. Moreover, general agreement was reached between OPP and the Services that it is unlikely OPP can develop specific testing methods to measure the effects of such mixtures in a quantifiable manner.*

Mixture Toxicity in the Context of Estimated Environmental Concentrations

The National Water Quality Assessment (NAWQA) Program (USGS, 2006) tested 51 major hydrologic systems across the U.S. with sampling locations selected to represent the land-use settings of greatest significance to these water resources, including agriculture, urban, undeveloped, and mixed. Water samples were analyzed for 75 pesticides and 8 degradates, including 20 of the 25 most heavily used herbicides, and 16 of the 25 most heavily used insecticides. Pesticides or their degradates were frequently detected in the more than 4,000 water samples. However, detection limits ranged from 0.001 – 0.240 µg/L or ppb (or 1-240 ppt), and typically were below 0.01 µg/L. The report states "pesticide occurrence in streams and ground water does not necessarily cause adverse effects on aquatic ecosystems or humans." Not surprisingly USGS found that "the pesticides detected most frequently [in streams and ground water] were among those used most heavily during the study period or in the past." While the USGS report hypothesizes on how mixtures can be dealt with in a more simplistic manner, the authors also recognized the challenges of designing methods to deal with this variability:

> *. . . the large number of chemicals and varying exposure routes that occur in the environment make testing every possible exposure scenario impossible. For example, in a mixture of 20 compounds, there are 190 pairs of compounds, and more than a million possible combinations (pairs, triples, and so on)."*

The USGS data show that while the occurrence of pesticide mixtures in surface waters across the U.S. is common, the levels detected are quite low and the specific components of the mixtures are quite diverse. The USGS data also show that the specific composition of pesticide mixtures most frequently found in water differs significantly according to land use and individual water bodies. Thus, defining a standardized surrogate mixture of pesticides or reliable procedure for toxicity testing that is environmentally relevant is beyond the practical power of currently available scientific methods.

The data for both humans and other species suggest that the vast majority of the time, when chemicals are present in a mixture at sufficient levels to cause toxicity, the

interaction is additive or less. The USGS data show that the vast majority of the time, concentrations of pesticides in the nation's waters are below aquatic benchmarks. Studies show that simply adding toxicity values for each chemical in the mixture can overestimate the toxicity. Further, if only one compound is present at toxic levels and the others are not, studies have shown that this compound will drive the toxicity of the mixture; so assessing the risk of the mixture based upon the toxicity of the driving compound is the currently the best available method for assessing mixtures of such low concentrations. The prevailing scientific opinion is that the methodology for risk assessment of mixtures is still under development and certainly is not mature enough to act as the foundation for regulatory decisions. Therefore, EPA's methodology of risk assessment and regulatory decisions based upon a single pesticide is appropriate and relevant given the concentrations routinely observed in the nation's waters.

1.7 Status of Exposure Modeling

The EPA OPP is required by FIFRA to assess the risk posed by pesticides to human health and the environment. The *hazard* data generating process discussed in Chapter 1 and the *exposure* considerations addressed in Chapter 4 are combined through modeling or expert opinion in determining what degree of *risk* is faced by a given group of organisms. OPP EFED is charged with carrying out the environmental portion of this assessment and does so by utilizing validated exposure models applied in a sequential fashion to the risk assessment process. There are many models available, but not all meet the requirements for relevancy and validation when applied in the environmental risk assessment process. Terrestrial models are just now emerging and are limited but of some usefulness in the environmental risk assessment process. There is a scarcity of models for assessing exposure in urban settings or other non-crop settings. Validated and reliable models have been developed for estimating exposure, and thus risk, to aquatic systems and organisms. The state of best available science/data for these models is reviewed below.

Terrestrial Models

Exposure modeling in terrestrial environments is dependent upon toxicity endpoints and estimated dietary exposure. For avian species, residues found or estimated on dietary food items following pesticide application can be compared to toxicity values to evaluate risk. For small mammal species, acute toxicity values are converted to estimated dietary toxicity values and compared to food consumption rates and estimated residue concentrations on food items. This is presently the best available methodology for approaching exposure modeling in non-target terrestrial organisms.

Terrestrial Vertebrates

Granular or Bait Formulations

EPA assumes that birds and mammals can be exposed to granular or bait formulations in direct relation to the amount of active ingredient applied per square foot of treated soil. For example, broadcast baits or granular soil insecticides are assumed to be completely

unincorporated. So, if a granular formulation was applied at 1 lb a.i./A, EPA would calculate that there are 454,000 mg a.i./A divided by 43,560 sq ft per acre or 10.4 mg a.i./A. EPA assumes that a bird or mammal would forage exclusively in the treated area and could ingest these granules intentionally, perhaps mistaking them for grit or seeds, or ingest them accidentally during the course of feeding.

In contrast to broadcast treatments, granules that are applied in a band or in-furrow are assumed to be mostly incorporated into the soil and thus not available for ingestion. As default values, EPA assumes that 85% of granules that are applied in a band are covered with soil, and that 99% of granules that are applied in-furrow are covered with soil. However, recall that EPA assumes that a bird or mammal forages exclusively in the **treated area.** Typical band treatments are 7" wide, centered on the seed furrow, and typical in-furrow treatments are 1" wide, and EPA would assume that birds or mammals forage only on these treated areas.

Granular insecticides are typically applied once per season, at planting time. EPA does not assume any degradation or disappearance of the active ingredient, so the amount of pesticide per square foot is only used to estimate environmental concentrations for acute effects.

At present, there is no standard model or procedure for deriving higher tier deterministic EECs for granular or bait formulations. In practice, the registrant might elect to gather additional data, for example on the degree of incorporation for their particular granule. Or, the registrant may elect to perform pen studies to evaluate the actual degree to which birds or mammals ingest the granules. ECOFRAM did propose a probabilistic model for granules called GEM (Granular Exposure Model) (ECOFRAM, 1999). It does not appear that this model has been implemented by EPA for regulatory decision-making.

Liquid Formulations

EPA uses a different approach for estimating environmental concentrations that result from applications of liquid formulations. It is based on the work of Hoerger and Kenaga (1972). These authors reviewed hundreds of studies on pesticide residues that result on crops immediately after application. The dataset was used to estimate residues on the following groups of bird and mammal food items: short grass; tall grass; broad leaf plants/small insects, and; fruits/pods/seeds/large insects. Note that the Hoerger and Kenaga dataset did not include actual measured residues in small or large insects; these values were assumed based on the size and shape of some of the plant matrices measured.

To allow for differing application rates, Hoerger and Kenaga developed the concept of the Residue Unit Dose (RUD) that results from application of 1 lb a.i./A. Residue estimates for lower or higher application rates are arrived at by linear interpolation of the Residue Unit Dose. Fletcher *et al* (1994) reviewed the Hoerger and Kenaga database and other studies and found that it generally captured the range of residue values observed. Fletcher *et al* (1994) did suggest slight adjustments in the Residue Unit Doses for some of the food groupings. In an assessment, EPA uses the maximum RUD, which is

designed to capture the upper 95% level of residues immediately after application. For risk characterization, EPA assessors may also consider the mean Residue Unit Dose. As an example, the maximum Residue Unit Dose for fruits/pods/seeds/large insects is 15 ppm, and the mean Residue Unit Dose for fruits/pods/seeds/large insects is 7 ppm.

In contrast to the situation for granules and baits, EPA does assume a half-life of pesticide residues on contaminated avian or mammalian food items. Absent valid residue decline data from the registrant, EPA assumes a half-life of 35 days for all food groups. This value was obtained from a publication by Willis and McDowell (1987), who tabulated measured residue half-lives from hundreds of rate of decline studies. The maximum residue half-life ever measured in the Willis and McDowell paper was 36.9 days.

The Estimated Environmental Concentrations above are used in EPA's TREX model. TREX is a spreadsheet based model that calculates estimated residues on food items and estimates pesticide intake. It assumes that a bird or mammal takes 100% if its diet from a treated field, and that residues are at the maximum. It also assumes the maximum application rate, the minimum interval between applications, and the maximum number of applications allowed per year. With this information, it calculates either 'dose-based' or 'dietary based' ingestion. As an example, a dose-based ingestion estimate for a small bird assumes it consumes more than its bodyweight in food per day. In all cases, the peak observed residue or rate of ingestion is used as the benchmark to compare to the lowest acute or chronic study endpoint.

EPA's procedures certainly overestimate actual exposure in the diet, because: (1) not all food items will can be contaminated at the maximum level; (2) a compound will not typically be applied at the maximum rate the maximum number of times, at the minimum interval allowed between treatments; (3) birds or mammals will not take 100% of their diet from a treated field; and (4) the half-life of the compound will likely not be 35 days. Furthermore, the comparison of the peak estimate of ingestion provides a further level of conservatism. This peak value, which occurs for 1 day, is compared to acute endpoints from a 5-day exposure in the avian acute dietary study. The 1-day peak is compared to the NOEC from a 20 week avian reproduction study. Proprietary field studies, submitted on various products, have shown that actual concentrations on bird or mammal food items in the environment are typically lower than the Hoerger and Kenaga maxima.

At present, there is no standard higher tier model for deriving deterministic estimated environmental concentrations. In practice, the registrant might choose to gather additional compound-specific data for input into TREX. For example, studies to measure the residue levels in bird or mammal food items could be performed. Also, given the sensitivity of TREX outputs to half-life values, the registrant might elect to perform studies to measure the half-life of a compound in bird or mammal food items. In its report to the Scientific Advisory Panel in March of 2000, EPA did propose a 4 tier process for higher level assessments. This process was directed towards probabilistic assessments and in general would require considerable new research and data development to implement.

Plants

EPA uses a series of assumptions to arrive at Estimated Environmental Concentrations (EECs) for plants. Spray drift from ground and aerial applications is assumed to be 1% and 5% respectively. Run-off from treated fields is arrived at based on the solubility of the pesticide. Up to 5% of the applied pesticide is assumed to run-off if solubility is greater than 100 ppm in water. EPA also assumes that pesticide from 1 acre of treated land moves by sheet run-off into 1 acre of adjacent terrestrial environments. EPA further assumes that pesticide from 10 acres of treated land moves by channelized run-off to 1 acre of semi-aquatic environments. All estimates assume a single application at the maximum label rate and do not allow for degradation. The results of this estimation procedure are compared to results from greenhouse studies. In practice, no commercially viable herbicide passes an assessment based on these estimated environmental concentrations, yet plant damage on off-target landscapes rarely occurs at an observable level, suggesting that the plant model is excessively conservative.

At present, there are no procedures, either deterministic or probabilistic, to develop higher tier EECs for non-target plants.

Exposure modeling in terrestrial environments is dependent upon toxicity endpoints and expected dietary exposure. For avian species, residues found or expected on dietary food items following pesticide application can be compared to toxicity values to predict hazard. For small mammal species, acute toxicity values are converted to expected dietary toxicity values and compared to food consumption rates and expected residue concentration on food items. This is presently the best available methodology for approaching exposure modeling in non-target terrestrial organisms.

Aquatic Models

To assess the risk to aquatic life posed by each chemical, the same exposure-to-hazard ratio is used as an indication of potential ecological risk to non-target species in the environment, but unlike the terrestrial situation, there are validated models in use to estimate exposure. The estimated environmental concentrations produced by the models can then be compared to the concentration that would be of concern (“level of concern”) for the organism being assessed in order to provide a context for a decision about risk.

Before the introduction of efficient models and the tiered risk assessment process, a “standard pond” calculation was used but was relatively labor-intensive and therefore time consuming. Consequently, a 'trigger' or 'screening' mechanism was used to establish which chemicals were most likely to pose higher risk and should be assessed in this manner. The screening mechanism established was termed the "Back-of-the-Envelope" calculation and was based largely on the solubility of the chemical. With the advent of better models and doubts about the usefulness of a chemical's solubility as a relevant screen, work was begun in 1994 to develop a new screen that would be more consistent

with other modeling approaches and better represent the parameters that are linked to pesticide transport to and persistence in surface water.

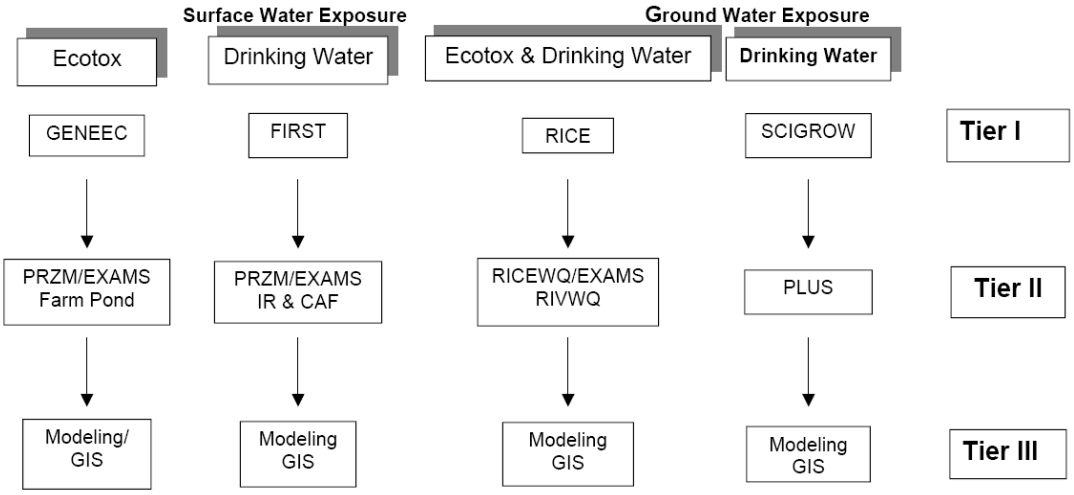
EFED has been performing pesticide aquatic exposure assessments as a part of the ecological risk assessment process for a number of years. Exposure assessments draw on both measured pesticide quantities in the field as well as computer modeling to establish the concentration levels which might be expected in significant aquatic habitats. With the development of enhanced environmental fate and transport models such as the Pesticide Root Zone Model (PRZM) (Carsel et.al., 1984, 1997), Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) (Leonard et al., 1989) and the EXposure Analysis Modeling System (EXAMS) (Burns et.al., 1982; Burns, 2000), computer modeling of pesticide exposure began to play a larger role in EFED's risk assessments in the early 1990's.

The triggering or screening level method originally used with the “Back-of-the-Envelope” standard pond calculation evolved as modeling progressed. It is generally agreed by risk assessors that it is appropriate to define a tiered process for when given models should be applied to the risk assessment process. In taking a tiered approach to refinement, it is not appropriate to use early-tiered modeled concentrations as the basis for national or regional exposure estimates. These models are useful as a “first cut” to determine if refined analysis is necessary. “Passing” a given assessment tier and not having to move on to the next indicates that OPP is confident that there is no potential risk or concern. “Failing” an assessment tier *does not* mean the chemical is likely to present a risk. Rather, it simply means that a small possibility exists that an extreme exposure could result in a risk quotient greater than the LOC, and the risk assessment should continue on to the next higher tier. As a matter of policy, OPP does not take significant regulatory action based on the results of screening models unless the model shows that there is no concern for assessing the risk further.

The following tiered structure is currently used at EPA for regulation of crop protection products (see Figure 1.3).

- Tier I uses simple, highly conservative models that are generally fast to run and require minimal compound data. Examples of models utilized at the Tier I stage include GENEEC, FIRST and Sci-Grow
- Tier II uses deterministic models that require far more data than Tier I models and take a longer time to run. Examples at Tier II include PRZM3, EXAMS and Leachm
- Tier III utilizes more advanced and customized analysis of exposure parameters, most often by spatial analysis using geographic information systems (GIS).

Figure 1.3. Examples of the tiered modeling process used to assess surface water and ground water exposure (Jackson and Gustafson, 2003)



The risk assessment process anticipates that it generally is not possible to model certain circumstances, such as for inert compounds or mixtures beyond Tier I, due to a lack of proper characterization data. When an organic (xenobiotic) compound such as a crop-protection product is developed for use, a set of standard laboratory tests is conducted to help predict compound behavior in the environment. In addition to the studies discussed above on the toxicity and sublethal effects of compounds, additional primary goals for environmental fate studies include determining how long compound degradation would take under the various conditions of use, and determining how degradation occurs.

Relevant scenarios and the tiered approach to modeling are designed to minimize the amount of analysis required to evaluate any given chemical. Each of the tiers is designed to reduce uncertainty by requiring higher, more complex levels of investigation only for those pesticides that have not passed the previous tier. Advancing to a new tier is no longer necessary when the risk quotients (RQs) fall below the levels of concern (LOCs), thus eliminating the need for a more rigorous review prior to the final risk management decision.

One important factor in developing exposure estimates is estimating uncertainty due to model inputs. This is particularly important, since it is known that standard EPA models typically provide estimated concentrations that greatly exceed measured levels, sometimes by several orders of magnitude. This inherent over-estimation provides a built-in conservative approach, which in turn manages uncertainty issues such as those related to the impact of mixtures or inerts, or to exposure input parameters such as intake rates, or the need to advance to higher tiers of modeling when no risk is indicated at a lower screening level. Models used in this process and how they are applied are discussed below.

Use of Models

The use of models to estimate such things as chemical fate and transport or an organism's exposure to a chemical is generally accepted within the scientific community. An explanation of the tiered assessment approach and the uncertainty inherent in the predictive nature of the models is provided here to increase understanding of how such models are used by EPA in the risk assessment process. EPA (USEPA, 2006b) notes that:

> *The two surface water models, Pesticide Root Zone Model (PRZM) and Exposure Analysis Modeling System (EXAMS) . . . have been extensively validated and peer reviewed. . . Additionally, when questions or potential enhancements are identified relative to the models used in pesticide ecological risk assessments, it is EPA's standard practice to vet these through the FIFRA Scientific Advisory Panel (SAP). The SAP is a committee chartered under the Federal Advisory Committee Act (FACA) and is comprised of independent scientists who consider pesticide scientific issues and advise the Agency regarding those issues. EPA's ecological risk assessment process uses output from other models and these models have also undergone rigorous validation and independent peer review. . .*
>
> *Through the use of models, which are based on real-world information, the Agency can estimate pesticide concentrations on a daily basis in any location of interest. The selection of input parameters and assumptions in these models are designed to significantly minimize the potential of underestimating likely exposure levels in the more vulnerable water bodies. Thus, although the models may not provide exact predictions of true exposures, it is highly unlikely that models will underestimate risk (and in some cases, the models will overestimate risk) for vulnerable sites. The estimates produced for vulnerable scenarios would likely be higher than the potential exposure for other, less vulnerable sites.*
>
> *Monitoring studies can tell us what is happening under current use practices and under a limited set of conditions. They are also useful in demonstrating trends of pesticide occurrence in a particular area of the country. Monitoring studies, however, do not provide a reliable estimate of acute exposure at vulnerable sites and do not capture the enormous variability that models can capture in terms of geographic location, timing, pesticide usage, and environmental conditions that exist throughout the country.*

Chapter Two: Extent and Quality of Data

2.1 Rationale for Use of Registrant ("GLP") Data

In the risk assessment process, EPA utilizes primarily the studies conducted in compliance with Agency guidelines and submitted by the registrant as required under FIFRA. These studies are high quality and designed to be the principal data set for conducting ecological risk assessments (including endangered species analyses). Guideline studies are conducted under strict Good Laboratory Practice (GLP) standards

including: validated testing methods; well characterized test substances; detailed testing protocols; a high level of documentation and independent quality assurance; and other features. These studies are subject to the rigorous acceptance criteria prior to their use in risk assessment and, once accepted, are considered validated and reproducible. Each study undergoes thorough review before being considered acceptable for use in risk assessment. Although the guideline data are likely to be the "best scientific ……data available" due to the stringent data demands of FIFRA, neither EPA nor the Services can "ignore credible available biological, toxicological or chemical information" from other sources. However, data from other sources must be subject to quality criteria regarding how the data were generated and the intended use or purpose for the data – in this case regulatory decision-making.

EPA pesticide testing guidelines are the basis for the regulatory acceptance of the concordant studies. The formal scheme of acceptance criteria applied when studies are peer reviewed by EPA scientists is as follows:

> *Core guideline*: Includes and meets all parameters and conditions specified in the guidance document for the given study.
>
> *Core minimum*: Includes and meets most of the parameters and conditions specified in the guidance document. Any deviations or omissions are not critical to the accuracy and validity of the resulting data.
>
> *Supplementary*: The parameters or conditions of the study vary from the guideline specifications to such a degree that the study cannot be considered core data but it is useful data. This category is also used for studies having no specifically developed test guideline, and in some cases for published data submitted to support data requirements.
>
> *Upgradeable:* The study is missing an element of supporting data, but the omission can be cured by the submission of additional data or documentation.
>
> *Invalid:* The test parameter or conditions are so far from those required as to invalidate the data for regulatory use.

EPA also uses the term "reserve" if an assessor concludes that an additional study listed in the full set of data requirements is believed not to alter the conclusions of the risk assessment should the study be conducted. If a new use scenario or other concern renders the missing information critical to the interpretation of risk, then the data requirement will be reconsidered.

Figure 2.1 illustrates the process for selection and use of guideline data and the additional data described in the next section.

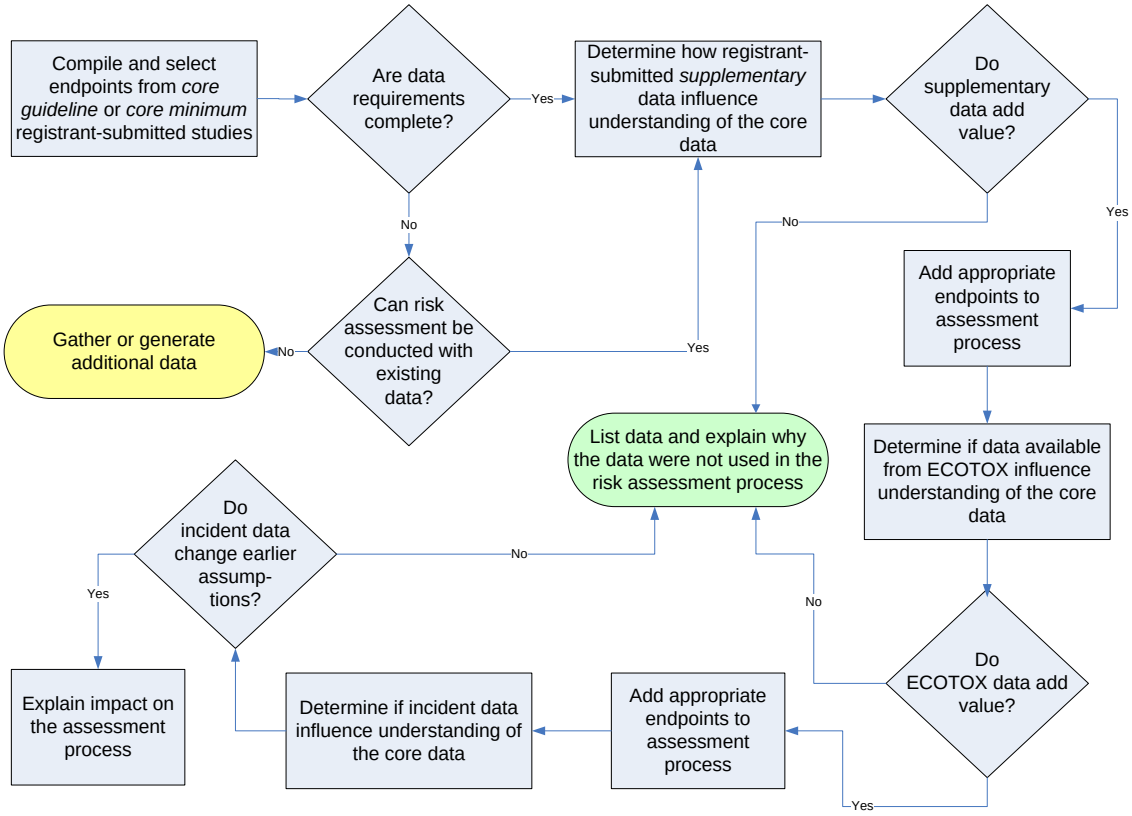
2.2 Rationale for Selection, Standards and Use of Additional Data from the Open Literature

While the peer-review process confers some credence to the information contained in a journal article, it does not necessarily bestow equal quality of such data to data developed under FIFRA and GLP guidelines, nor does it indicate that data from a peer-reviewed article is of high enough quality or contextually relevant for regulatory decision-making. For example, peer-reviewed articles often do not include analytical verification of the test substance; the dose selection may be inappropriate for developing a dose-response curve or the determination of a no effect level; the exposure conditions may not mimic nature; there may be inconsistencies in the presentation of the data; and the endpoints selected may or may not have bearing on ecologically relevant effects. These types of studies are not designed for purposes of risk assessment or regulatory decisions and should not be relied upon as a substitute for or an alternative to FIFRA guideline directed studies. In the EPA Guidelines developed pursuant to the Office of Management and Budget's (OMB) data quality guidelines, assessment factors have been established for evaluating the quality and relevance of "external" sources of data. These criteria, which serve as the building blocks for data quality, (see Figure 1.2, section 1.2) are:

1. **Quality.** Quality is an encompassing term comprising the other criteria below. The aggregate quality of the individual studies, methods and other information supporting an assessment must be considered.
2. **Utility.** Utility refers to the usefulness of the information to its intended purpose and users, including the public. The various studies, data, sources of information and methods that potentially could be used in the assessment must only be relied upon to the extent that their use can be scientifically justified to the degree the design of the study or method affords.
3. **Objectivity**. Objectivity includes whether disseminated information is being presented in an accurate, clear, complete, contextually appropriate and unbiased manner. Beyond presentation, the sources of the disseminated information (to the extent possible, consistent with confidentiality protections) must be identified including the supporting data and models.
4. **Transparency**. Uncertainty and error sources affecting data quality should be identified, documented and disclosed. A high degree of transparency about data and methods (source, assumptions, statistical methods, criteria for study acceptance, scientific justification for use of data/methods) will facilitate reproducibility of such information by qualified third parties.
5. **Quantity**. The magnitude of the effect, numbers of studies, the number of high quality data points, the number of replicates and sample size or power must be considered.
6. **Consistency**. This criterion refers to the extent to which similar findings are reported using similar and different study designs and methods. Consistency applies to both studies and analyses used in the assessment.
7. **Integrity.** The integrity criterion refers to scrutiny, such as the protection of information from unauthorized access or revision, to ensure that the information is not compromised through alteration or improper interpretation.

Quality criteria underpinning "Best …available" must also apply to incident data[2] that is considered to any degree in the assessment process. Incident reports are often speculative allegations that are of no value to risk assessment and often do not generally contain data that meet the requirements of "best scientific……..data available." Such reports can only be useful in providing information on the field effects of pesticides when the quality of the incident reports and their content actually have been verified.

Figure 2.1. Proposed rationale and documentation of the selection and use of scientific references (data) in the risk assessment process



2.3 The Role of Benefits Data

Many products, such as those available for wetland and rangeland recovery, reforestation or weed control on idle land, are used to restore or protect the environment and as such bring benefit to the environment. Non-native pests are the source of an estimated $120 billion annual economic burden to the United States and are the second leading cause of species endangerment (Pimentel *et al*, 2004). In addition, Huber, *et al* (2002) reports that bioterrorism introduces another unknown risk factor into the already unstable mix of detrimental exotic species. The effects brought on by invasive species include loss of power; loss of farmland; depreciation of property value; contamination of grain for export; spread of disease; increased cost of operation; decreased efficiency of production

[2] From the Environmental Incident Information System, maintained by EPA OPP.

and irrigation; economic annihilation of agricultural producers; collapse of buildings; competition with native species; loss of sport, game, and endangered species; and disturbance of ecosystems. There is a growing danger to national forests, recreational, and agricultural lands from invasive plant infestations. These infestations are causing costly and irreparable damage to wetlands, wildlife habitat, wildlands, rangelands, and aquatic and riparian areas on public and private lands all across the United States, especially in popular western recreation states.

The U.S. Geological Survey reports that some $20 billion in damages can be linked to invasive plants alone. The infestation rate on federal lands is now approximately 4,600 acres per day (Asher and Harman, 1995); that is invasive plant species spread to infest an additional three million acres every year, an area twice the size of Delaware. Invasive plant species represent a form of biological pollution that disrupts the function of ecosystems on a landscape scale and such alterations multiply what were once unique regional characteristics resulting from thousands of years of natural selection to a blur of wide changes that result in the loss of decades of conservation achievements. When left uncontrolled, spreading invasive and noxious weed species produce many direct and secondary negative effects, including the creation of plant monocultures that negatively impact endangered species and crowd out desirable native species and forage used by game and livestock through direct competition in the ecosystem and by allelopathic poisoning of the soil. These impacts also reduce rangeland productivity, increase livestock production costs, and increase soil erosion in addition to degrading the aesthetic qualities of the land. Unchecked, invasive species threaten the reliability of utility power lines, increase the need for railroad and road maintenance, and increase fuel availability for wildfires. Approximately 46% (Wilcove et al, 1998) of all federally listed endangered species are critically imperiled by invasive species. Collectively, these impacts can severely interfere with recreational activities in parks, forests and other public lands, resulting in reduced property value, erosion of local tax bases and impaired local economies.

Western states have the highest numbers of ESA listed species that need relief from stressors of this type. Examples include millions of acres of leafy spurge and spotted knapweed on western rangelands; the parasitic plant witchweed, which threatens important agronomic crops across the southern United States; and the recent invasion of giant salvinia, which poses a severe ecological and economic threat to the lower Colorado River system (Mullin *et al*, 2000).

FIFRA procedures allow the consideration of the benefits of pesticide use, but ESA procedures do not. A large portion of the target organisms that prompt a pesticide application, however, are invasive species. Invasive species displace native species, especially endangered species because they are those most vulnerable to stressors. This creates an entirely different risk assessment paradigm than simple pest control within a crop habitat. The basic risk assessment premise used to evaluate a crop herbicide against the FIFRA standard of "no unreasonable adverse environmental effect," is that no environmental degradation will result if the product is not used. That is, any negative effect would be limited to a weedy field, which while costly to the farmer, does not qualify as an adverse environmental effect. However, that assumption is not valid when

assessing those herbicides designed for invasive and noxious weed control. The risk assessment premise for exotic invasive plant species must consider the invasive plant as an unnatural state with an impact on the ecosystem that is similar in many aspects to a disease epidemic in an animal population. The use of an herbicide product for invasive and noxious weed control must be assessed in light of ecosystem degradation if no action is taken. When considered together, invasions by exotic plants, animals, and pathogens, are regarded by biologists as one of the major threats to biological diversity worldwide. Consequently, in denying the use of an herbicide for the control of invasive species, the agency taking such steps may actually be promoting what the ESA describes as a "take."

Consequently, in making a decision on stressors, the benefits of pesticides must be considered. In the problem formulation process, and/or in the discussion of stressors, one question that needs to be considered is whether or not the pesticide under evaluation will be used in such a way as to relieve the effect of other stressors on non-target species. One of the central precepts of the Endangered Species Act is whether or not "an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species." (50 CFR 402.02) Therefore, *not* taking a positive registration action for a product which can eliminate stressors that are reducing the reproduction, numbers, or distribution of a listed species could in itself be introducing jeopardy to listed species. Therefore the risk assessor must structure the risk assessment to inform the risk manager whether their *registration action* will have an adverse effect on any listed species.

Chapter Three: Addressing Complex Endpoints: Process Development in Emerging Science

Regulatory agencies have recognized the complexity of dealing with environmental variables and that the conservatism built into the risk assessment process is the only currently available method to deal with various uncertainties such as species spatial and temporal status, environmental contaminants in existing habitats, and the effect of multiple stressors on an organism or population. In a point-specific assessment, such as one conducted for a building site, these factors are more identifiable, but on a national scale assessment, such as that required for a pesticide registration action, it is impossible to describe all the conditions in all of the areas where a pesticide may be used. It then becomes important to address those elements that are most significant in the assessment of risk and that can be addressed at the national level, moving to more specific geographic analysis when necessary to draw a sound scientific conclusion.

3.1 Environmental Baseline

The Services are charged with describing the "environmental baseline" for a given species and action. National level "baselines" however, simply do not yet exist. On a national scale, the elements selected for review of the environmental baseline by sheer geography and number would be subject to selective and incomplete description and would not provide the context required for a discussion of the baseline adequate to allow

a determination as to whether a pesticide use produces a significant and measurable direct or indirect effect when compared to the stressors that could occur in any situation, anywhere in the nation. The NOAA Fisheries Northwest Region has developed an ESA consultation initiation template to "encourage consistency in format and content, to reduce our additional information requests and to facilitate and expedite our reviews." (US NOAA, undated). In this document, the environmental baseline is described as follows:

> *This section is an analysis of the effects of past and ongoing human and natural factors leading to the current status of the species, its habitat (including designated critical habitat), and ecosystem, within the action area. The environmental baseline is a "snapshot" of a species' health at a specified point in time. It does not include the effects of the action under review in the consultation.*
>
> *The baseline includes State, tribal, local, and private actions already affecting the species or that will occur contemporaneously with the consultation in progress. Unrelated Federal actions affecting the same species or critical habitat that have completed formal or informal consultation are also part of the environmental baseline, as are Federal and other actions within the action area that may benefit listed species or critical habitat.*
>
> *The environmental baseline should paint a picture of the habitat for listed or proposed species in the action area and amount of degradation that has occurred to date. Describe the present condition of the habitat elements essential for the listed or proposed species. If the action area includes designated critical habitat or proposed critical habitat for the listed species, describe the critical habitat and level of degradation.*

The suggestions in this guidance highlight the intent that the baseline be built around a local and temporal, site-specific circumstance. A good example of how this can be done is presented by the Services in the Rock Creek Mine Biological Opinion (Services, 2006). Scientific methodology simply does not exist for expressing a baseline on a national level. The aggregation of site-specific information into a national data set, and the effort to temporally place it for every possible pesticide application, would exceed the resources of any regulatory agency in the nation. However, it is of value to develop a general baseline that considers what the stressors are to a listed species, and how the use of a pesticide affects or compares with them. For example, an important aspect of the baseline for a listed aquatic plant may be competition from invasive exotic species. Thus the registration of an herbicide that controls the invasive species improves the condition for that species rather than degrading it. It may be possible to develop key items such as this that serve as bellwethers in lieu of the formal and daunting task of attempting to establish a national baseline.

For pragmatic purposes, in the pesticide risk assessment process the potential risk of a pesticide is assessed in a highly conservative, protective manner, thereby allowing for

what might be the impact of other stressors. For this reason, Williams and Hogarth (Services, 2004) concluded:

> *. . . the Services and OPP have agreed that OPP's risk assessment process will use the following methodology when developing an environmental baseline. Consistent with 50 C.F.R. 402.14, developing an environmental baseline is only necessary where OPP has determined that a pesticide action likely will adversely affect a listed species, or will destroy or adversely modify critical habitat. In such instances, OPP will ask the Services to provide appropriate information concerning the status of the species and other information necessary to establish an appropriate environmental baseline.*

In this way, site- or temporally-specific data can be provided by the Services to EPA, or gathered by EPA from other sources, to determine whether there is indeed a risk posed to the organism and site in question. To a great extent, the FIFRA Endangered Species Task Force (FESTF) Information Management System serves to define a baseline on a national scale, by identifying where there is an intersection between a species and use, and by providing, over time, retrievable information on local circumstances or relationships between the species and potential use sites.

3.2 Cumulative Effects

The challenge of scientifically and meaningfully defining and qualifying "cumulative effects" is that this would require definitive predictions of the future actions of a State or private citizens. In fact, Williams and Hogarth (Services, 2004) recognize this in their consideration of this topic and EPA/Services interactions on endangered species assessment:

> *A cumulative effects analysis is required for Federal actions likely to adversely effect listed species. See 50 C.F.R. 402.14(c). Within the biological opinion, it commonly is the least documented part of any effects determination, given the lack of definitive information on future State and private activities. See Consultation Handbook, Procedures for Conducting Consultation and Conference Activities Under Section 7 of the Endangered Species Act, Page 4-30 (FWS and NMFS, March 1998).*

Thus, on a national (and even local) level, the assessment of cumulative effects must be more qualitative than quantifiable. Additionally, ESA Section 7 regulations require the federal action agency to provide analysis of cumulative effects *only* when requesting the initiation of formal consultation. It is also important to note that the broadness or narrowness of the definition for "cumulative effects" varies between environmental statutes. For example, "cumulative effects" when used in the FIFRA (or more specifically the Food Quality Protection Act) context means the additive effect of molecularly related active ingredients. In ESA definitions, "cumulative effects" means the effect on species of future State or private activities, not involving federal activities, that are reasonably certain to occur within the action area subject to consultation (Services, 1998). The National Environmental Protection Act defines "cumulative effects" as the impact on the

environment which results from the incremental impact of the action under evaluation when added to other past, present and reasonably future actions regardless of what agency or person undertakes such actions (CEQ, 1997)

The Services concluded (Services, 2004):

> ***Where OPP has determined that an action is likely to adversely affect listed species or result in destruction or adverse modification to critical habitat*** [emphasis added], *OPP will consider cumulative effects as part of its effects determination. This analysis will be based upon the best scientific and commercial data available. The extent of this analysis will vary depending upon the level of concern initially identified and the geographical reach of the action area.*

3.3 Contaminant Load

As discussed in section 1.5, predictions of the environmental concentration of a single pesticide are used in the pesticide risk assessment process. However, there is no validated and reliable method for predicting the level and type of contaminants that *might* occur in any given circumstance. Contaminant load is in fact a more detailed evaluation of the elements of a baseline, and thus less likely to be quantifiable even on a local basis. The conservatism built into the prediction of exposure and interpretation of hazard, when applied to the pesticide risk assessment process, is currently the only method available to provide a protective margin to account for such uncertainties as contaminant loading, and thus the best available approach to the issue of contaminant load.

3.4 Action and Action Area

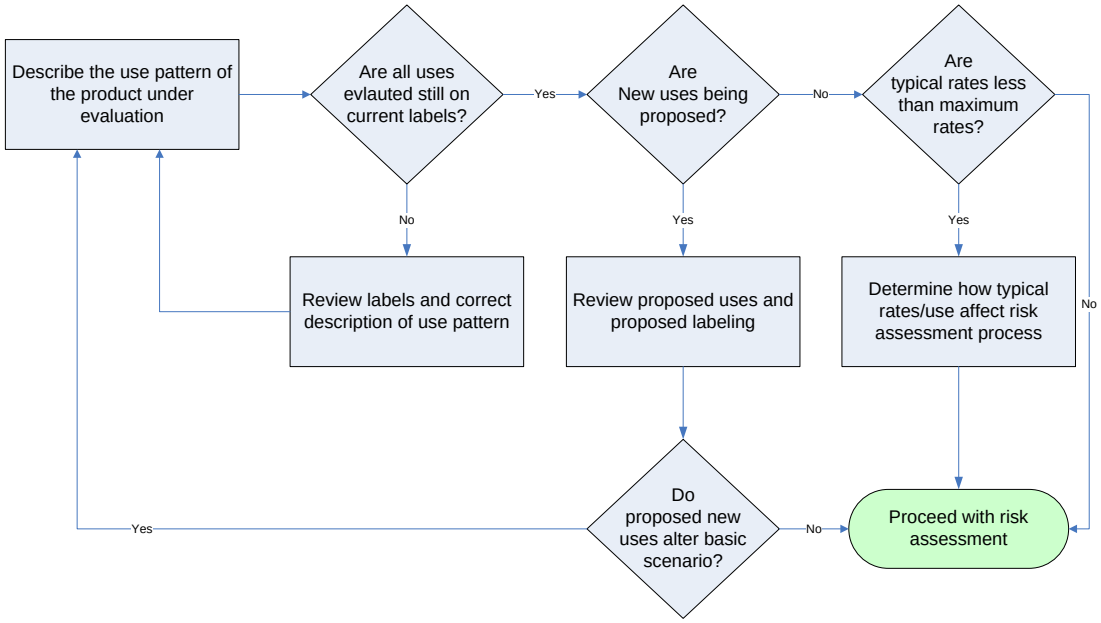
The action area as defined in the Consultation Handbook translates to an expression of all places where a permitted pesticide use *could* take place and *might* affect a listed species or its habitat. From the point of view of actually protecting a species, and rationally defining potential exposure, this area can be winnowed down through a series of logical steps. For a national pesticide registration, a tiered approach to the refinement of the action area may be necessary, in a manner similar to the approach to modeling aquatic concentration of a pesticide for risk assessment purposes. While it is not currently scientifically possible to individually assess every geographic location where a pesticide may be used, it is possible to first start by narrowing all possible agricultural areas with a national evaluation of where the pesticide might be used based on the spatial distribution of the use site (corn, for example). Given this subset of locations, the area of interest within the action area can be further defined by environmental parameters that preclude the use of the pesticide under evaluation (soil types, agronomic practices, or other environmental factors). Further refinement of areas of interest within the broad action area, if necessary to examine a potential risk situation, can be accomplished by spatial analysis of the overlap between the refined areas and the known locations of the sensitive area or species. This can be done at a low level of resolution using the federal or state geographic data sets, or at a higher level of resolution (resulting in a smaller and more precise area of overlap) using land cover and hydrologic data. Thus, the action area is defined broadly but refined for assessment in an iterative process, starting on a national

level based upon potential use and narrowed as relevant chemical specific information is acquired, and the problem formulation (discussed below) is refined.

3.5 Problem Formulation

The Problem Formulation schematically documents the structure of the risk assessment process. Minimally, it should include the chemical description, a conceptual model of the routes and amounts of likely exposure, a definition of the action area (based on labeling or spatial analysis), and any uncertainties that could lead to an assessment that overstates risks and leads to unnecessary restrictions. Current EPA risk assessment processes are extremely unlikely to ever *underestimate* risk. Label summaries support many aspects of the assessment, including determination of uses that define the action area, selection of exposure scenarios for consideration in the assessment, refinement of exposure estimates for uses relevant to the species of concern, and development of mitigations and protections focused on specific labeled uses. Assessing labeling before beginning the risk assessment is important to proper definition of the problem (see Figure 3.1).

Figure 3.1. Preparing for the Risk Assessment Process



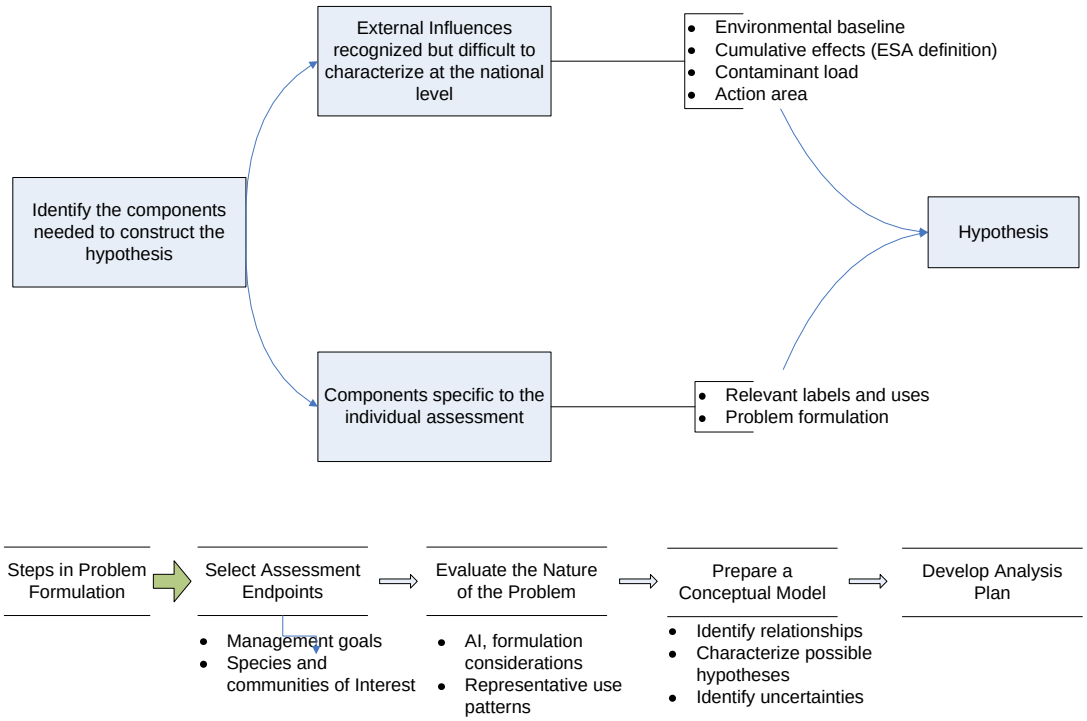
There are currently two schools of thought on the role of a problem formulation: (1) the problem formulation establishes a proposal for how the risk assessment will be conducted and what the underlying assumptions are in the risk assessment process; or (2) a lengthy and thorough retrospective description of what was done in the risk assessment process. EPA has provided a technical overview that supports the development of a problem formulation as a tool to define the analysis plan (USEPA, 2006a). EPA notes that risk managers first discuss their risk management goals, options to achieve those goals, and the scope and complexity of the risk assessment prior to entering into the problem formulation process. After this phase,

> *. . . the risk assessors and risk managers perform the following tasks:*

- ***Select assessment endpoints*** *- these are based on management goals identified in the planning dialogue and are important because they provide direction and boundaries for the risk assessment. Assessment endpoint include two elements: identification of the specific ecological entity that is to be protected, such as a species, a community, an ecosystem, or other entity of concern and a characteristic about the entity of concern that is important to protect. In a screening-level pesticide ecological risk assessment, typical assessment endpoints are reduced survival and reproductive impairments for individual animal species. For plants, the assessment endpoints are typically concerned with maintenance and growth of non-target species. Although these assessment endpoints are measured at the individual level, they indicate potential risk to populations.*
- ***Evaluate the nature of the problem*** *- this step includes defining the nature of the stressor (pesticide) and characterizing the pesticide use. In defining the nature of the stressor, risk assessors generally focus on the pesticide active ingredient although in some cases they may consider pesticide formulations, inert ingredients, or degradates based on available data. Risk assessors use the pesticide product labeling to characterize the nature of the pesticide use in the field. Characterization of pesticide use allows the risk assessors to focus the risk assessment on specific use patterns that are representative of a larger variety of use patterns. In this way, risk assessors can focus on use scenarios that reasonably represent the highest exposures.*
- ***Prepare a conceptual model*** *- the conceptual model includes a set of risk hypotheses and a diagram that describe the predicted relationships among stressor, exposure, and assessment endpoints. Typical conceptual models are flow diagrams that contain boxes and arrows illustrating these relationships. Developing a conceptual model allows the risk assessor to identify the available information regarding the pesticide, justify the model, identify data and information gaps, and rank model components in terms of uncertainty.*
- ***Develop an analysis plan*** *- this is the final stage of problem formulation in which risk assessors develop a plan for analyzing data and characterizing risk. The analysis plan summarizes what has been done during problem formulation and targets those hypotheses that are likely to contribute to the risk. It also evaluates the risk hypotheses to determine how they will be assessed, develops the assessment design, identifies data gaps and uncertainties, determines which measures will be used to evaluate the risk hypotheses (e.g., LC50, NOAEC, EEC's), and ensures that the planned analyses will meet the risk managers' needs.*

The above components are diagrammed in Figure 3.2. The science of risk assessment is an evolving one, and the approach to problem formulation is an area that is currently receiving a good deal of attention. As definition is assigned to this element of the risk assessment process, risk assessors will adaptively manage their approaches to meet best available documentation and methodology.

Figure 3.2. Risk Assessment: Constructing the Hypothesis



Chapter Four: Common Foundations in Combined FIFRA and ESA Assessments

4.1 Impact of the Active Ingredient Alone or As a Full Strength or Diluted End Use Product

The risk assessment process includes steps to characterize the possible exposure to the environment from inert and other ingredients (adjuvants, impurities, and degradates) found in, added to, or formed from crop protection end use formulations. In the hazard data development scheme required by FIFRA, both the active ingredient and end use formulation are subject to acute mammalian toxicity testing. Additionally, if there are any impurities or degradates of toxicological concern, FIFRA requires the registrant to either provide data on these materials, remove them, or reduce their concentrations below levels of concern in the final pesticide formulation. The risk assessment characterizes the risks associated with these materials and the final formulation (see Figure 4.1).

The role of company risk assessors and managers is key to synthesizing important additive facts as data on a given active ingredient are developed. Industry expertise is used to address: toxicity by different routes of exposure (for example, use of mammalian data to determine if there is any evidence of enhanced toxicity based on exposure route); toxicity of formulations (review of components of the formulation, or comparison of

acute tests with representative formulations to those on the technical to determine if there is an indication of enhanced toxicity); potential toxicity of mixtures (results from biological studies with the target pests); significance of degradates (a scientific assessment of which degradates occurring at 5-10% of the parent should be tested), and; the consideration of the presence of impurities (testing is done with typical technical product, which should have all impurities). The company will conduct additional mammalian studies if they perceive a potential problem. EPA will also make these considerations as the data are reviewed and assessed.

The process of considering whether special attention needs to be given to the inert ingredients or impurities in an end use formulation, as well as the need to address degradates of the active ingredient that result upon storage or environmental degradation, is discussed below.

Assessing risk on active ingredients and end use formulations

In the guideline testing procedure, key studies are run on the active ingredient, and the final formulation is subjected to mammalian acute toxicity testing. Should any significant toxicity differences, on a pure active ingredient basis, be noted, the Agency may require additional testing of the formulation, or may reject the composition of the formulation. In these acute tests, the materials tested are required to fully represent the final labeled product, meaning that any additives or impurities in the final product are included. EPA makes a separate determination on whether the impurities or additives present an unexpected hazard, and has standards for restricting the presence of certain impurities that are known to be of concern (certain dioxin molecules or hexachlorobenzene (HCB), for example).

Results of the tests on the technical product or "active ingredient" can be compared to toxicity results for the formulation to assess whether or not the formulation toxicity differs from the active ingredient toxicity. If the formulation exhibits greater toxicity on an active ingredient equivalent basis than the active ingredient alone, then the risk assessor considers how the formulation endpoints affect the selection of toxicity values for calculation of the risk quotient. This can be done by using the formulated product toxicity data as a definitive endpoint in the risk assessment process, or by considering the order of magnitude of increased toxicity of an end use product compared to the active ingredient toxicity, given equal concentrations of the toxicologically active component.

Globally, there are variations in data requirements, some of which include ecotoxicological testing of end use formulations in aquatic species, or more rarely in avian species. While these studies are not always submitted to EPA, companies are required to report studies to EPA where unexpected adverse effects are observed for US-registered products under the requirements of FIFRA 6(a)(2).

It is uncommon for a formulated product to display greater non-target toxicity than the active ingredient, particularly when one considers the dilution of the material in a final spray mixture and ultimately, the dilution that can occur when released into the

environment. Acute oral studies on mammals and birds use undiluted test material, unless dosing requires the aid of a carrier such as water or corn oil. This exposure to "in the can" material would only occur in the environment in the event of a spill or flagrant misuse of a product in undiluted form. Products that are applied directly from the container, such as home pest control aerosols, are packaged in small containers and already diluted.

Unlike active ingredients, inert ingredients are often mixtures, making certain property determinations difficult or even impossible to define, which is expected to limit the accuracy of model predictions for such materials. Therefore, the conservatism build into risk assessment procedures, with respect to the use of hazard and exposure data in the modeling process, has been designed to deal with these uncertainties.

Assessing risk related to impurities and degradates of toxicological concern

EPA requires the thorough investigation of any impurity or degradate of toxicological concern. Impurities arise in the manufacturing process and can consist of artifacts related to incomplete chemical reactions, reagents used in the manufacturing process, combustion or heat-related byproducts of the reaction process or other fragmented or altered molecules. The registrant is required to analyze the components of a final technical product by identifying all materials present at a level of 0.10% or higher. If any of these materials are of a structural chemical composition expected to produce toxicity ("of toxicological concern"), EPA may require separate testing to evaluate whether such impurities need to be removed from or reduced in the technical product or final formulation. A judgment on "toxicological concern" is accomplished by comparing the chemical structure of the impurity to the parent compound or through the evaluation of chemical structure-activity relationships using software programs that allow molecules to be drawn in 3-D renderings, which are then compared to a library of compounds with similar structures and known physical, chemical and toxicological properties. This evaluation allows the scientist to predict the potential toxicity of a compound based upon the known toxicity of other compounds, because it is a basic tenet of toxicology that chemicals with similar molecular structure typically have similar toxicological effects.

In a similar fashion, breakdown products that may be produced in the environment are evaluated. Environmental fate pathways and mechanisms are fully explored through required studies on the biological and physical breakdown of active ingredients under both laboratory and field conditions. Like impurities, degradates of toxicological concern that might be produced are subjected to further evaluation or testing and these results are considered in the risk assessment process (see Figure 4.1). Furthermore, the requirements of 6(a)(2) also apply to the discovery of adverse effects from degradates or impurities of toxicological concern. Varying global data requirements can generate additional data and if that data is adverse, it must be submitted to EPA for products registered in this country. Adverse data discovered and submitted in this manner may trigger the need for additional testing to clarify the presence or degree of an actual effect.

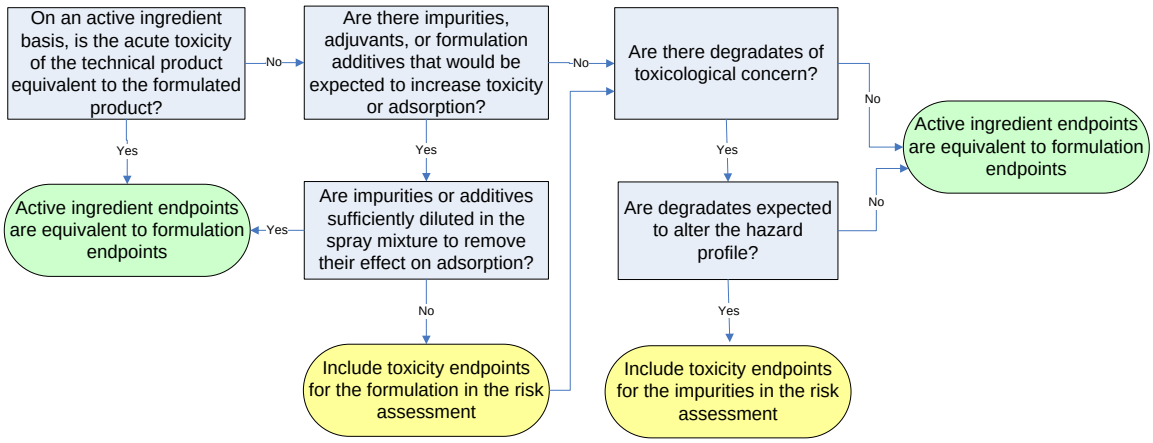
With this background, it is useful to visit the Services summary of the handling of formulations, inert ingredients and degradates in the EPA FIFRA testing and risk assessment process (Services, 2004):

> *OPP conducts a screening level assessment on a formulated product only if data is available indicating the formulation is more toxic than the active ingredient. OPP would expect to receive such information pursuant to section 6(a)(2) of FIFRA, which obligates registrants to submit any information related to known adverse effects to the environment caused by a pesticide. OPP's approach differs from procedures followed within the European Union (EU), only to the extent that the EU requires registrants to submit acute toxicity data on the product formulation for mammals and aquatic fish and invertebrates if initial tests on the active ingredient suggest concerns. Because OPP would expect data required by the EU to be reported to OPP under section 6(a)(2) if the tests involve a formulation that is approved in the United States and the data indicate greater toxicity than other available studies, OPP's process ultimately would be expected to produce largely comparable data as the data used by the EU. To the extent additional and accessible data exist, however, OPP has agreed to obtain and consider such information in its risk assessment process. Additionally, OPP's review of the open literature as described above, will be expected to identify other available data addressing the effects of pesticide formulations.*
>
> *Because new environmental fate and toxicity data specific to product formulations will not always be available, OPP's process for examining formulated products is less robust than the process used to assess active ingredients. The Services conclude, however, that OPP's existing process for evaluating formulations makes use of the best scientific and commercial data available. Beyond this, the Services note that EPA is engaged in several efforts designed to improve its assessment of formulated products. Under FIFRA, EPA is engaged in a Congressionally mandated, multiple year review of all food use inert ingredients, to verify safety determinations previously made by the Agency under the Federal Food, Drug and Cosmetic Act. Internally, OPP is actively reviewing in-house ecological effects databases, to summarize information on the distribution of the relationship between the toxicity of active ingredients and formulated products.*

Steps in the assessment process to address the impact of the active ingredient alone or as a full strength or diluted end use product

In order to ensure that inert ingredients, impurities and degradates are considered in the risk management decision, the risk assessor addresses the properties of the active ingredient and formulated product in a logical manner to determine if special considerations are necessary to adjust the assessment of risk for these factors. A schematic of this process is shown in Figure 4.1.

Figure 4.1. Proposed steps to assess the possible variances of toxicity, exposure or risk of an active ingredient when formulated as an end use product



4.2 Use of Surrogate Species to Establish Toxicity to Multiple Taxonomic Groups

Toxicity data are not available for every taxonomic group that might be exposed to a broadly used product, and it is not feasible to test every species. Surrogate species data must be used when a risk assessment is conducted on species for which no toxicity data are available. Surrogate species toxicity data are the best available data as the basis for the assessment. However, the uncertainties in extrapolating from surrogate species to listed species must be understood, and are often addressed in risk assessment through the use of safety factors (Chapman *et al*, 1998; Duke and Taggart, 2000). In OPP's pesticide assessment process (USEPA, 2004a), the safety factors are implemented as Levels of Concern (LOCs).

The standard surrogate species tested for pesticide registration include freshwater and marine fish, invertebrates, and algae; marine mollusks; freshwater and terrestrial vascular plants; birds; and mammals. Most of these categories include two or more standard test species, and the most sensitive species is used as the basis for risk estimation. Many of the standard test species, including rainbow trout, *Daphnia magna*, and mysid shrimp, are consistently among the most sensitive species to pesticides and other chemicals. Use of these species as surrogates for their respective taxonomic groups is therefore protective of a large fraction of the species in those groups (Giddings *et al*, 2000).

In OPP's risk assessment process, species that may be more sensitive than the surrogates are protected through use of a safety factor applied in the form of an established LOC for acute and chronic risk to each taxonomic group. The acute LOC for listed aquatic animal species is 0.05, equivalent to a 20-fold safety factor applied to the surrogate species toxicity value. The 20-fold safety factor accounts for variation in sensitivity among individuals (as represented by the acute dose-response relationship) as well as variation in sensitivity among species. As discussed in Section 1.5, dose-response curves for most pesticides are steep enough that even a 2- or 3-fold reduction in dose reduces the likelihood of individual mortality substantially. Most of the 20-fold safety factor therefore accounts for interspecies variation, i.e., for the possibility that a listed species is

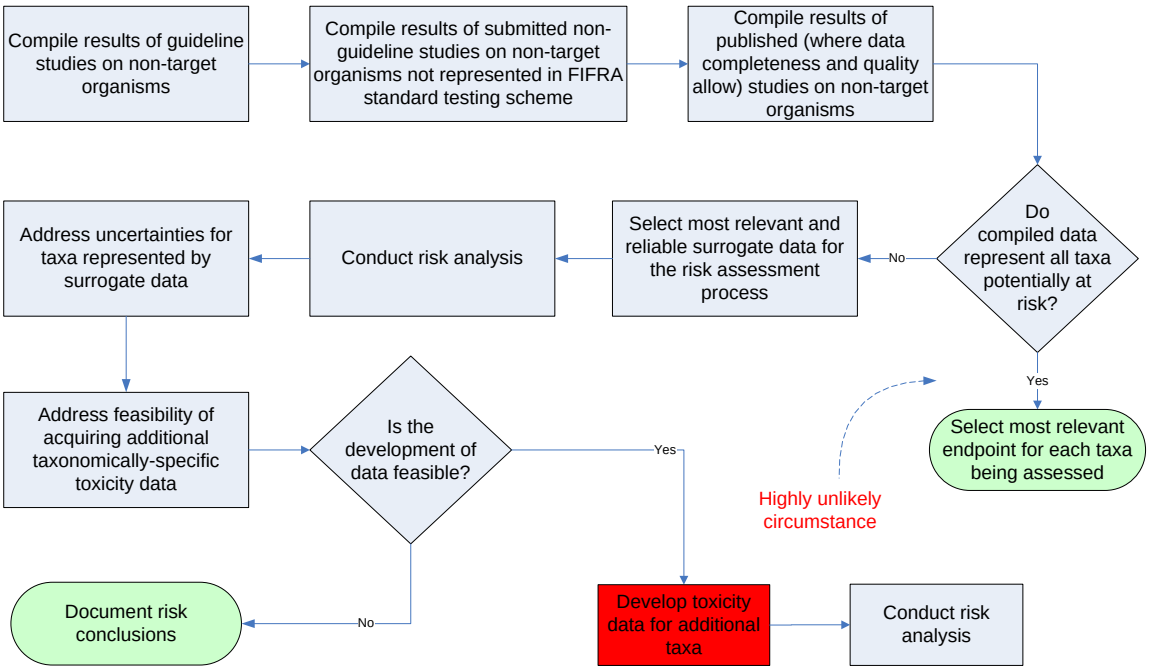
substantially more sensitive than the most sensitive surrogate species in the taxonomic group. In a similar fashion, a safety factor of 10 is applied to avian species acute toxicity values.

The LOC for chronic effects on listed and non-listed species is 1. No safety factor is applied because the toxicity value used in the assessment is the No Observed Effect Concentration for the most sensitive test species. In cases where chronic toxicity data are unavailable for sensitive species, EPA may use an Acute-to-Chronic Ratio (ACR) to estimate chronic toxicity based on acute toxicity to a sensitive species. The ACR is calculated from measured acute and chronic toxicity values for other species. When data are scarce, an ACR of 10 is assumed (USEPA, 2004). The chain of assumptions and extrapolation factors for chronic effects may introduce an intolerable amount of uncertainty into the risk estimation, in which case EPA normally requires that studies be conducted to fill chronic toxicity data gaps.

In a few cases, the sensitivity of threatened and endangered species has been measured and found to be similar to that of standard surrogate species. Sappington *et al* (2001) tested standard fish species (rainbow trout, fathead minnow, and sheepshead minnow) and endangered species (Apache trout, greenback cutthroat, Lahontan trout, bonytail chub, Colorado pikeminnow, razorback sucker, Leon Springs pupfish, desert pupfish) by exposing them to carbaryl, permethrin, and 3 non-pesticide toxicants using standardized water quality and test conditions. The results were compared within fish families. Salmonids, represented by the rainbow trout, were the most sensitive fish family, a finding also reported by others (Macek and McAllister, 1970, Mayer and Ellersieck, 1986, Beyers *et al*, 1994). In only two cases did a listed species have a 96-h LC_{50} that was less than one half that of its surrogate. Fairchild *et al* (2008) conducted acute and chronic toxicity tests with rainbow trout and bull trout (a listed species) with 3 rangeland herbicides and found that the two species were similar in sensitivity (acute and chronic toxicity values within a factor of 2). While such data are sparse, they are consistent with trends shown by analysis of much larger datasets for aquatic species (Asfaw *et al*, 2003) and wildlife (Raimondo *et al*, 2007a,b) in which interspecies toxicity correlations were found to be strongest for species most closely related taxonomically.

Use of surrogate species toxicity data requires an evaluation of the characteristics and magnitude of the uncertainties inherent in the use of such data. The assessment process under FIFRA includes a 10-fold safety factor for the acute risk to endangered terrestrial animal species and a 20-fold safety factor for the acute risk to endangered aquatic animal species. In the documentation of the assessment, the assessor should evaluate these safety factors with respect to major sources of uncertainty in the process before drawing a final conclusion about risk. Figure 4.2 illustrates the sequence of steps in the evaluation process.

Figure 4.2. Proposed steps to extrapolate toxicity to multiple taxonomic classes of organisms



In the Services' review of EPA's risk assessment practices (Services, 2004), Hogarth and Williams note that

> *. . . while this surrogate information is the best available toxicological data, OPP's analysis will discuss species extrapolation uncertainties to ensure that scientific judgments using this data are made in a transparent manner. The Services and EPA will work cooperatively to develop methods in the future to increase confidence in the use of surrogate species test data, such as determining whether new safety factors may be identified, or exploring opportunities for testing additional species. . . OPP will use methods described earlier concerning "Best Scientific and Commercial Data Available" to ensure that any additional information is obtained.*

An example of EPA's discussion of the uncertainties of extrapolating from surrogate species to listed species can be found in a recent EPA effect determination for the California Red-legged frog ("CRLF," USEPA, 2008):

> *Guideline toxicity tests and open literature data on propyzamide are not available for frogs or any other aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern;*

however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

4.3 Estimating and Simulating Exposure

Because every circumstance and every species cannot be analyzed or predicted, the risk assessment must depend upon a certain degree of extrapolation and exposure simulation. Extrapolation and simulation are initially based on findings generated by controlled laboratory studies or rigorous field studies, both of which are designed to remove or deal with environmental variables in such a manner as to prevent interference with the interpretation of results. In the simulation process, conservatism is built into the assumptions made by selection of the most highly vulnerable conditions, such as:

- Static water bodies (no outflow); watershed area is high relative to volume of the water body
- The entire watershed is assumed to be cropped and treated
- Maximum label rates and minimum treatment intervals are used
- Heaviest historical rainfall and run-off conditions are selected
- Drift is considered to always be toward the non-target area of concern
- Annual application of the same product to the same location is assumed year after year
- Direct exposure is assumed to be to the highest labeled concentrations as the only circumstance (i.e. total diet is obtained from the treated field at the highest treated rate; total aquatic environment is limited to waters exposed to the product)

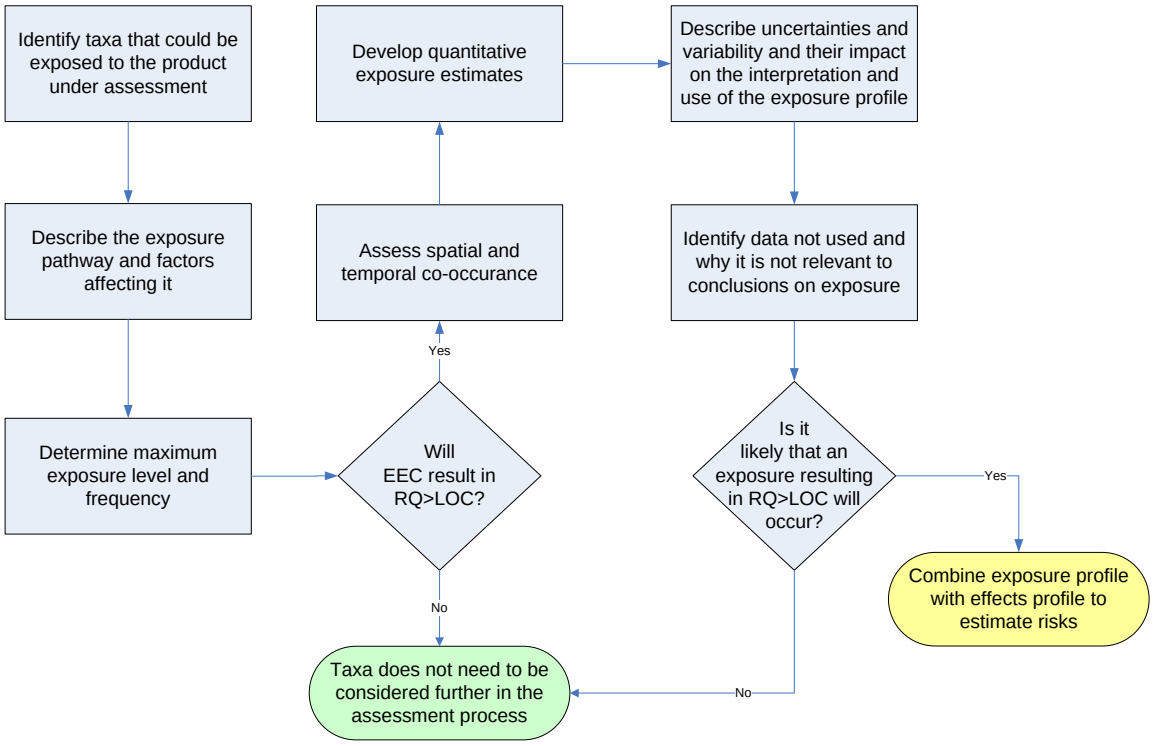
These "worst-case" assumptions imbue the assessment with a high degree of bias toward overestimating risk, thereby resulting in an extremely conservative analysis.

EPA notes that the objective for the characterization of exposure is:

> *. . . to produce a summary exposure profile . . .that identifies the receptor (i.e., the exposed ecological entity), describes the course a stressor takes from the source to the receptor (i.e., the exposure pathway), and describes the intensity and spatial and temporal extent of co-occurrence contact. The profile also describes the impact of variability and uncertainty on the exposure estimates and reaches a conclusion about the likelihood that an exposure will occur.*
>
> *The exposure profile is combined with an effects profile . . . to estimate risks. For the exposure profile to be useful, it should be compatible with the stressor-response relationship generated in the effects determination* (USEPA, 1998).

The approach to constructing an exposure profile is presented in Figure 4.3 and discussed below.

Figure 4.3. Suggested approaches to exposure simulation in the development of an exposure profile



Exposure Routes

It is not possible to test every species for every route of exposure. Mammalian toxicity studies are generated via different exposure routes (oral intubation [oral insult], dietary, dermal, inhalation) and are used to determine if there is a quantitatively different toxicological response depending upon the route of exposure. Other routes, not expected in environmental exposure, are also sometimes tested (intravenous, interstitial, intraperitoneal). These routes bypass the organism's natural barriers resulting in an "internal" dose rather than a level of exposure or "external" dose, which is relevant to environmental exposure. And these studies are usually conducted to investigate a particular toxicological mechanism or metabolic function. Routes such as these are generally not relevant to environmental exposure and subsequent risk assessment.

Over the years, there have been attempts to establish new guidelines or protocols for examining additional routes of exposure in non-target organisms, for example dermal exposure to birds. To date, however, there is no validated and reliable method for the conduct of such studies, and in the occasional instances where such studies have been conducted, they generally yield no additional information that impacts the outcome of the risk assessment process.

Simulation of Exposure to Terrestrial Organisms

As Williams and Hogarth (Services, 2004) note,

> *Current analysis of terrestrial exposure either focus exclusively on dietary exposure, or expresses exposure on a generalized potential bioavailable mass of pesticide on a per unit area basis. The Services agree that the dietary exposure analysis is appropriate as a means of estimating dietary exposure. Potential exposure through inhalation or dermal contact currently constitutes an unknown for which the risk assessment provides no available information. OPP has developed proposals to analyze inhalation and dermal exposure for birds in such a way that it may be added to dietary exposure, and thus used in the development of a risk quotient. Similar proposals for other classes of species are expected in the future. The Services support the development and implementation of these proposals, following external peer-review by the FIFRA SAP. Pending this, however, the data on dietary exposure remains the best available quantified information provided through existing models.*

As scientific methods for developing exposure profiles advance and are validated, new approaches to simulating exposure will be adopted as the best available method for defining an exposure profile. Currently, the uncertainty of the contribution made by pathways not specifically included in the exposure profile is in part, if not wholly, addressed by the method used for estimates of dietary exposure. Exposure to terrestrial organisms is based on very conservative assumptions inherent in the EPA's terrestrial residue exposure model (TREX). Nonetheless, the representation of other routes has been and will continue to be explored. Company assessors are likely to make a reasoned argument about what additional routes of exposure should be tested based on their knowledge of the toxicology of the compound and its use pattern.

The EPA's effort to characterize exposure to terrestrial organisms via routes other than dietary is evidenced by proposals documented in the ECOFRAM terrestrial draft report (1999) and discussions with FIFRA Scientific Advisory Panel (SAP) (USEPA, 2004b). Specifically,

- Approaches/models to analyze terrestrial exposure via inhalation and dermal routes have been considered and are documented in the ECOFRAM terrestrial draft report (ECOFRAM, 1999).
- Models for dermal and inhalation exposures have been proposed and documented in EPA's discussion with FIFRA Scientific Advisory Panel – for example, see the Revised Terrestrial Level II Model (USEPA, 2004b).
- Inhalation rates key to estimating inhalation doses have been documented in the EPA's Wildlife Exposure Factors Handbook (USEPA, 1993).
- The work of Driver et al. (1991) has been considered by the EPA in their proposals to characterize exposures to avian species via routes other than dietary. However, Driver's work was with methyl parathion, a highly toxic and highly volatile insecticide. Similar work on other products requires

knowledge of the properties of the compound and a determination as to whether such testing would produce meaningful results.

Proposals have been developed to relate external inhalation dose to oral dose equivalents for avian species. However, combining overall ingestion, inhalation, and dermal doses to generate a total dose that would be useful in risk assessments is difficult for the following reasons:

- The fraction of the external dose that becomes available at a site or sites of toxic action within the organism probably differs between ingestion, inhalation and dermal exposure
- Although highly unlikely, the site or sites of toxic action may be different for different pathways (for example, irritation of gills in tadpoles as opposed to no toxicity to the terrestrial form of an amphibian when exposed dermally)

For these reasons, it would be difficult to generate a comparison of a total dose to dose-response data to generate a risk assessment. Consequently, until the emerging science discussed above is validated and reliable, characterization of exposure via the dietary route is the best available quantifiable information.

<u>Simulation of Exposure to Aquatic Organisms</u>

The science of predicting water concentrations has advanced somewhat further than terrestrial modeling, partly because the aquatic environment is more specifically definable. Currently, the majority of the national uses of every pesticide are fully analyzed with conservative models. As discussed in section 1.5, the tiered approach to refining aquatic exposure is applied to the development of exposure profiles for aquatic organisms. Williams and Hogarth (Services, 2004) note

> *For aquatic exposures . . . GENEEC2 is used as a rapid screen to separate low risk pesticides from those requiring more refined assessments. If "levels of concern" (LOC) for aquatic species are exceeded using GENEEC2 . . . EFED uses a second model known as PRZMS-3, which determines more site and use specific levels of pesticide in surface runoff. These runoff estimates are then further evaluated using a third model known as EXAMS II, which simulates the dissipation and degradation processes that occur in the water body to estimate daily pesticide concentrations.*

Additionally, EPA has demonstrated an approach to complex analyses of residential and commercial scenarios in recent reviews and it is recognized that the aquatic exposure estimation process will advance as new techniques are evaluated (Services, 2004):

> *The current model used to estimate aquatic exposure is intended to establish a "worst case" scenario, and therefore an upper bound exposure. Nonetheless, there are some unique scenarios in which even this model may underestimate exposure, due to specific circumstances (e.g. potential that a vernal pool may develop unpredicted high concentrations due to evaporation). Although there are no*

> *currently-established models that fully address these unique situations, OPP has developed proposals for aquatic exposure models for puddles, run-off, or similar exposure. The Services agree that these proposals could possibly be more effective at estimating some of these unusual exposure scenarios, and support OPP's intentions to incorporate these models in the near future, pending a favorable external peer-review by the FIFRA SAP. In addition, the Services are developing information that may be used in the future to further refine these models. In the meantime, however, the Services agree that the existing model necessarily represents the best available approach currently producing data for estimating aquatic exposure.* (Services, 2004)

The worst case scenario mentioned by Williams and Hogarth is developed by constructing a series of assumptions that individually could and collectively do exceed any expected "real world" conditions. In addition to conservative assumptions such as 100% of the planted crop is treated by the compound under assessment, modeling uses environmental loading factors from both runoff and drift with minimal account for degradation and dilution from the delivery point to the non-target water body. Standard environmental drift/aerial loading rates also assume that worse case climatic conditions apply for all applications, and that these conditions are often more extreme than conditions under which pesticide use is permitted by its labeling. The AgDrift model used for predicting movement and deposition by off-target drift is based on the most comprehensive drift study ever conducted, via an industry consortium and at a cost in excess of $20 million. The standard EPA assumptions on drift for multiple applications of a product further compound these conservative assumptions and therefore significantly (and purposefully) overestimate exposure.

The unique circumstances noted by Williams and Hogarth (Services, 2004), such as vernal pools, are not modeled separately, as they note. The models, though based on a static system, are also conservative for development of an exposure profile in flowing waters. Models such as GENEEC2 are conservative for sensitive species that live in flowing water systems since the actual residence/exposure times in flowing water will be much shorter. As noted by the Services, GENEEC2 does conservatively predict exposure for most aquatic situations by combining drift and runoff. For shallow water bodies that are typically generated by runoff, the combination of drift and runoff used in GENEEC will be overly conservative since both routes are unlikely to occur at the same. Additionally, ultra-shallow water bodies may only be utilized by a very small range of organisms, and during only a portion of their life cycle, and thus GENEEC2 is protective of most cases. Furthermore, because of the failure to cover mixing and flow, the standard methods fail to account for the existence of refugia. The impact of both the modeling conservative assumptions and the shortcomings of exposure simulation are considered and documented in the exposure profile development process, as depicted in Figure 4.3, above.

Chapter Five: Hypothesis Testing and Uncertainties

5.1 Defining the Context of a Determination

In 2000, EPA's Office of Science Policy published a handbook (USEPA, 2000) devoted to risk characterization. The foreword to the Handbook notes:

> *This Handbook was prepared by the Science Policy Council (SPC) for EPA staff and managers and others as a guide to Risk Characterization. It implements EPA's March 1995 Risk Characterization Policy which improved on the foundation of the February 1992 Agency-wide policy for risk characterization. Both the 1992 and 1995 documents point out that "... **scientific uncertainty is a fact of life (and) ... a balanced discussion of reliable conclusions and related uncertainties enhances, rather than detracts, from the overall credibility of each assessment** ...". **Both also note that while the role of science to inform but not make decisions is widely recognized in EPA, and in the larger risk assessment and regulatory community, these communities often use the risk assessment number as the stated reason for decisions, not always clearly highlighting the legal, economic, social and other non-scientific issues that also go into the decision.*** [emphasis added]

Very often the line between the scientific risk assessments and the risk management decisions based upon them becomes blurred. This circumstance is magnified when the basic premise for the risk assessments are not fully explained or when the risk assessor does not fully address the needs of the risk manager. A failure to communicate combined with professional differences of opinion exaggerates what in the end may be similar opinions.

Williams and Hogarth (Services, 2004) note that

> *The Services understand that legitimate professional differences of opinion may from time to time cause EPA to produce an effects determination that differs from that which a Service biologist might reach with the same information, just as two Service biologists may disagree on the interpretation of scientific data.*

The differences between scientists can be reduced by careful attention to a clearly defined risk hypothesis, an understanding and explanation of protection goals and a description of what constitutes "jeopardy." Each of these three areas is very broad and each are lacking in quantitative definition, which would help reduce the uncertainty in the risk assessment process.

Risk Hypothesis

In the context of some discussions, FIFRA's endangered species risk assessment process has been accused of having a bias toward erring in a way that causes a false negative (assuming no effect when one is actually possible) while the Services assessment process

functions in a way to cause a false positive (assuming an effect when one may not occur). NOAA Fisheries expressed this concern as follows:

> *To prevent* [jeopardy to species], *the Services must treat evidence and uncertainty differently than most other agencies: to minimize risks to listed species, we conduct our analyses and navigate our decision-making processes to avoid false conclusions at each step of a consultation . . . (that is, the Services are biased to avoid the "false negative" conclusion or minimize the risk of Type II error).*
>
> *Most other agencies, including EPA, conduct their assessments in ways that avoid concluding that agency actions had adverse effects when, in fact, such a conclusion is false (that is, they are biased to avoid the "false positive" conclusion or minimize the risk of Type I error).*

Statistical hypothesis testing is one of the most misunderstood and poorly expressed elements of statistical analysis. The description of error types as portrayed above is not a reflection of Type I or Type II statistical bias. Probabilistic predictions of an effect all have both Type I and Type II errors within them. To overcome the errors inherent in any predictive modeling procedure, the process and models used by EPA are built to generate concentrations higher than would be expected to occur in the environment. They are, by definition, screening level models. The actual risk to the organism is therefore reduced by over-estimating the levels of concentrations to which it may be exposed. In applying the screening level models to endangered species assessment, if a pesticide fails to "pass" the screen (that is, a risk is predicted) in this conservative process, then the resulting decision is protective, and leads to one requiring more data or further refinement beyond that provided by the first tier of the assessment process (Warren-Hicks, 2007).

Therefore, there is no statistical bias in the construction of the risk assessment process: that is not relevant to the overall assessment process. EPA utilizes the best available data and methods in constructing the risk assessment. Improvements in documentation, such as providing a more complete description of problem formulation, are currently being developed by to meet the risk characterization goals of transparency, clarity, consistency and reasonableness (EPA Office of Science Policy, 2006). In doing this,

> *Environmental risk assessments can provide high confidence of minimal risk by testing theories, "risk hypotheses", that predict the likelihood of unacceptable harmful events. The creation of risk hypotheses and a plan to test them is called problem formulation. Effective problem formulation seeks to maximize the possibility of detecting effects that indicate potential risk; if such effects are not detected, minimal risk is indicated with high confidence. Two important implications are that artificial test conditions can increase confidence, whereas prescriptive data requirements can reduce confidence (increase uncertainty) if they constrain problem formulation. Poor problem formulation can increase environmental risk because it leads to the collection of superfluous data that may delay or prevent the introduction of environmentally beneficial products* (Raybould, 2006)

Uncertainties

As noted above, uncertainty cannot be eliminated from a predictive process, but uncertainties do need to be recognized and identified to the risk manager in order to facilitate a sound decision. EPA notes (USEPA OPP, 2006b):

> *Uncertainties in estimating pesticide exposure can arise when using monitoring data as well as when using modeling estimates. Consequently, the Agency evaluates the uncertainties in the data and estimates associated with both types of approaches. By using multiple lines of evidence derived from monitoring studies and model predictions, the Agency can reduce the uncertainties associated with each of these approaches. Both modeling and monitoring estimates are important sources of information for characterizing exposure, and both methods typically provide complementary insights on the potential fate and transport of pesticides in the environment. For this reason, the Agency uses both simulation modeling estimates and monitoring data to characterize pesticide exposure and to produce scientifically robust risk assessments.*

Currently, there is no identified method for quantitatively characterizing uncertainty. The best available method is to apply statistical analysis where practical and possible and to provide qualitative description where otherwise appropriate. EPA instructs (EPA Office of Science Policy, 2006):

> *While it is generally preferred that quantitative uncertainty analyses are used in each risk characterization, there is no single recognized guidance that currently exists on how to conduct an uncertainty analysis. Nonetheless, risk assessors should perform an uncertainty analysis. Even if the results are arrived at subjectively, they will still be of great value to a risk manager. The uncertainty analysis should, in theory, address all aspects of human health and ecological risk assessments, including hazard identification, dose-response assessment, and exposure assessment. Uncertainty analysis should not be restricted to discussions of precision and accuracy, but should include such issues as data gaps and models.*

In reviewing EPA's procedures, Williams and Hogarth (Services, 2004) note:

> *. . . uncertainty would be highlighted, and if the risk manager determines that no adjustment needs to be made from the exposure levels otherwise estimated despite the highlighted uncertainty, this decision would be explained in the record of the risk assessment. By this method, OPP ensures that all available information will be considered, and data that are relevant, quantifiable, and reliable will be used.*

Addressing Protection Goals: ESA Jeopardy versus FIFRA Adverse Effect

In its Risk Characterization Handbook, EPA notes that risk assessment and risk characterization are not the same (EPA Office of Science Policy, 2000). How the risk relates to protection goals is different than simply addressing the numbers generated in the risk assessment process:

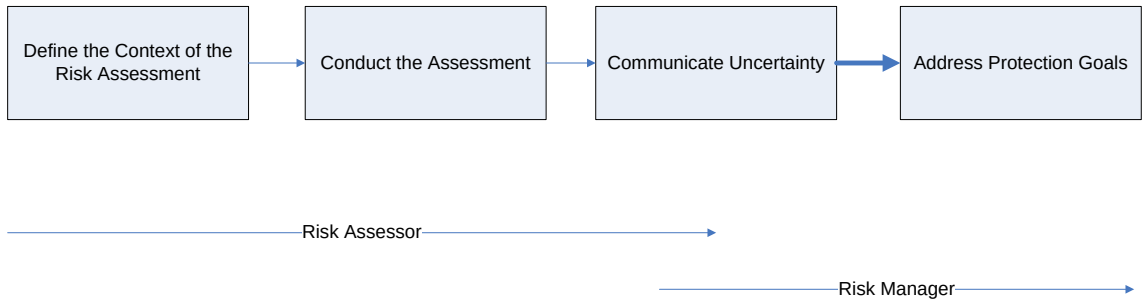
> *Risk assessment is a process comprised of several steps . . . Risk characterization is the culminating step of the risk assessment process. Risk characterization communicates the key findings and the strengths and weaknesses of the assessment through a conscious and deliberate transparent effort to bring all the* ***important*** *considerations about risk into an integrated analysis by being clear, consistent and reasonable.*

While EPA's FIFRA risk management goals are driven by the statue's "no unacceptable adverse effect," EPA enters an entirely separate process, with additional safety factors and at a higher tier, for addressing endangered species risk. The added safety factors used for listed species, and more rigorous investigation of species- or site-specific conditions, account for the ESA statue's stricter protection goals. EPA then characterizes the risk to listed species in making its risk management decisions.

5.2 Unifying the Approach and Protection Goals

The risk assessment process as discussed in this chapter follows the same constructs regardless of the driving Act. The basic goals of both ESA and FIFRA are met in the process described herein and simply illustrated in Figure 5.1. The foundation of the process, and the transition from risk assessment to risk management is accomplished under FIFRA in a way that meets the needs of ESA protection goals.

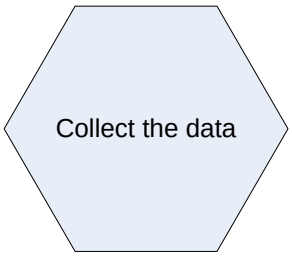
Figure 5.1. Risk Assessment: The Common Process



Chapter Six: Conclusion

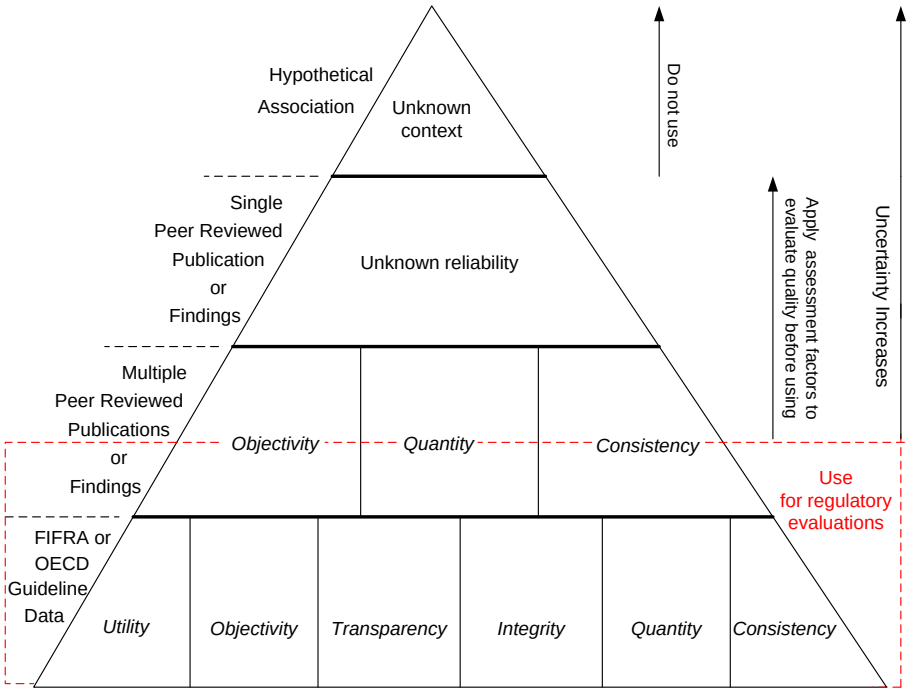
In conducting risk assessments, EPA utilizes the best available methods and data to characterize the risk presented by pesticides to endangered species. These methods meet the statutory demands of both FIFRA and ESA with respect to the protection of endangered species. The five modules discussed in the 5 chapters above define an approach to the risk assessment process. This process can be illustrated by combining the flow charts in each chapter to describe a comprehensive, thorough process that meets the transparency, clarity, conciseness and repeatable criteria of EPA risk assessment goals.

- ***First work module (Chapter1)***



Overview Document: IV.B; IV.C.2.a,c,d; IV.C.1; V.A.3; V.A.4.a,b,c; V.B.2.c,d; V.C.1,2; V.C.1,2,4.a,b; V.C.5; VI.A,B; VI.D.1-8; VI.E

- Utilize data that support the objectivity, transparency, integrity and consistency of the assessment



"Best available" loses reliability as it sheds its components of quality

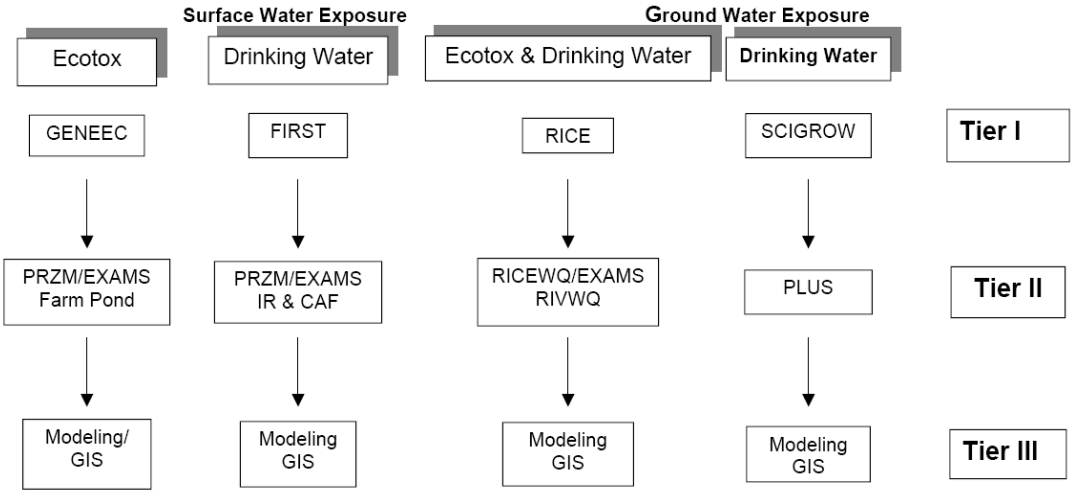
FIFRA Guideline Data: Has its *utility* defined by FIFRA testing guidelines; has its*consistency* defined by EPA review; has its *quantity* defined by FIFRA data requirements; has its *objectivity, transparency* and *integrity* assured by GLP requirements.

Repeated Peer Reviewed Publications or Findings*: Has consistency defined by replication; demonstrates quantity based on the statistical design of the studies; has its objectivity from peer review; has ultility to the extent it supports risk assessment; but loses its integrity and transparency because methods are not documented to the degree GLP's require.*

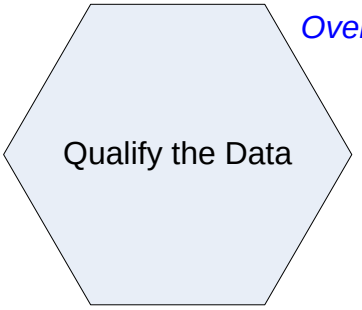
Single Peer Reviewed Publications or Findings: Has limited *objectivity* depending on the level of peer review, but has its *utility* defined by one circumstance and may not have been designed for purposes of risk assessment; and loses its *transparency b*ecause methods are not documented to the degree GLP's require, loses its *quantity* by its isolation and is of unknown *consistency*.

Hypothetical Association: Has virtually no *utility;* loses its *objectivity* to subjective speculation; has no *transparency* in methodological scientific application; is not supported by any *quantity* of data; and has no measure for *consistency*.

- Access appropriate data to reflect the level of refined assessment, and refine the assessment to best characterize the potential risk in a tiered fashion, for example

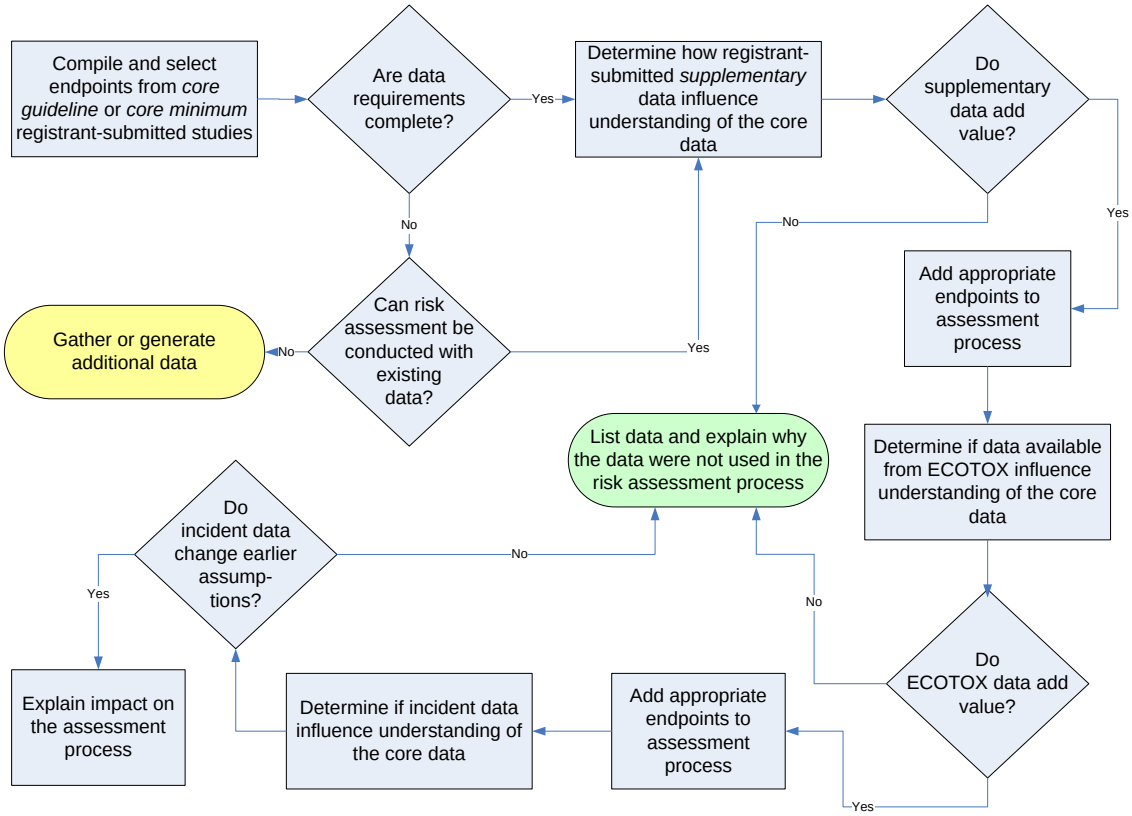


Second work module (Chapter 2)

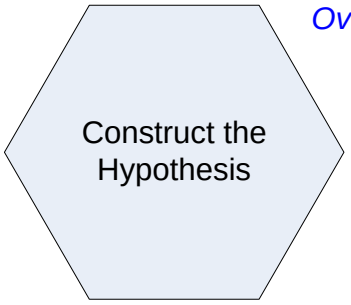


Overview Document: IV.A,B; IV.C.2.b; IV.C.3,4; V.B.1.a;V.B.2.a-d

- Use a logical progression of steps to select and rely upon scientific references, suggested here as diagrammed below

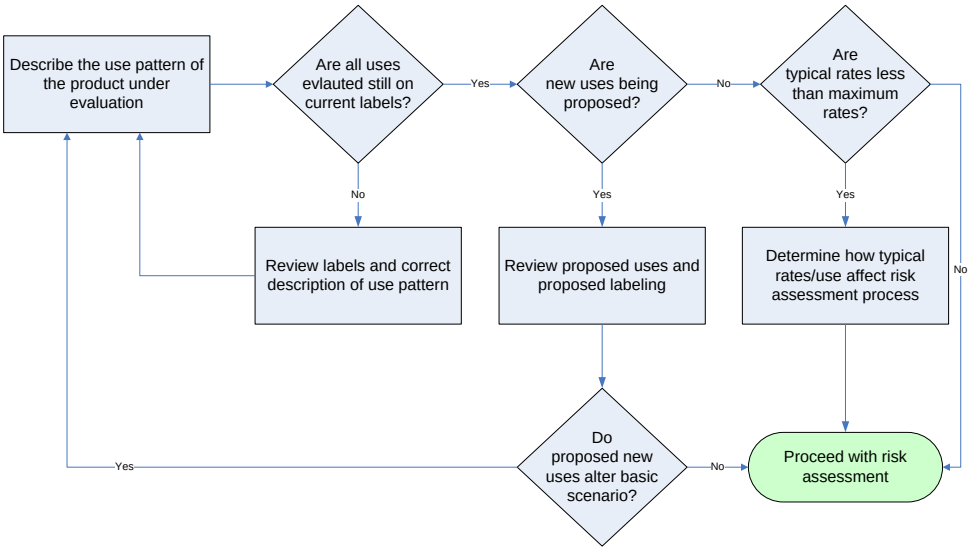


- ***Third work module (Chapter 3)***

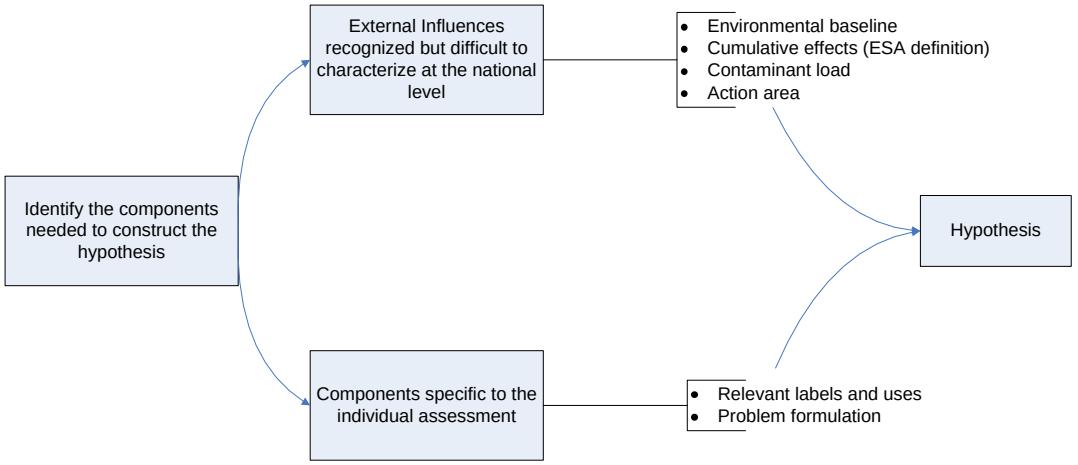


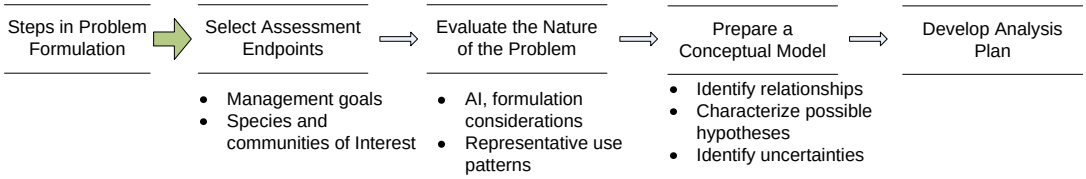
Overview Document: V.A; VI.H

- Ensure a valid basis for setting up the assessment

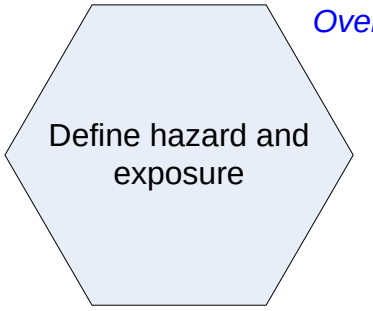


- Carefully construct the problem and hypothesis



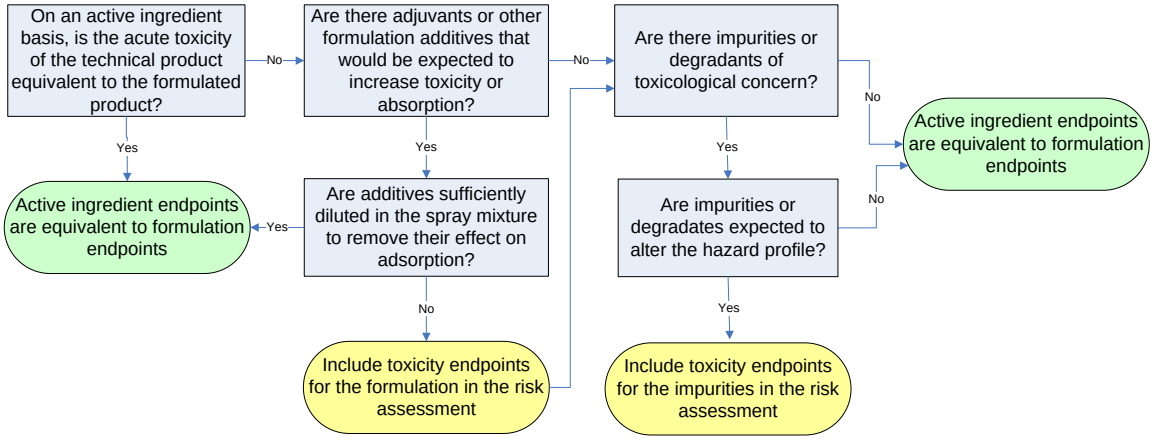


- ***Fourth work module (Chapter 4)***

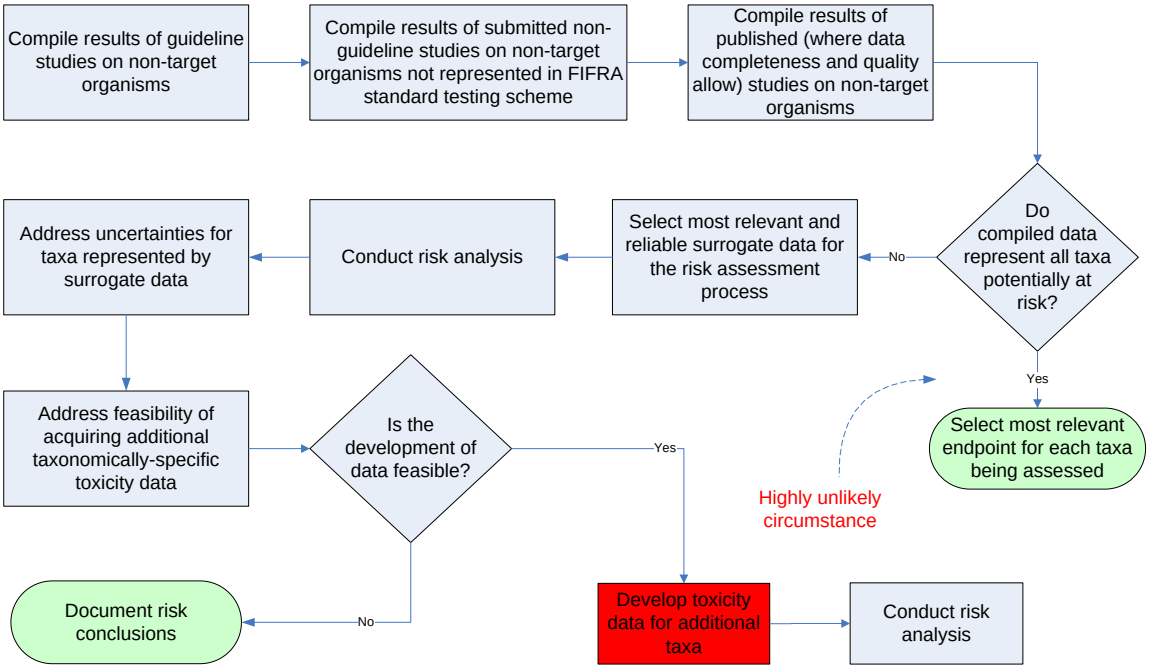


Overview Document: V.A.2,5,6 V.B.1.b,c,d; V.B.2

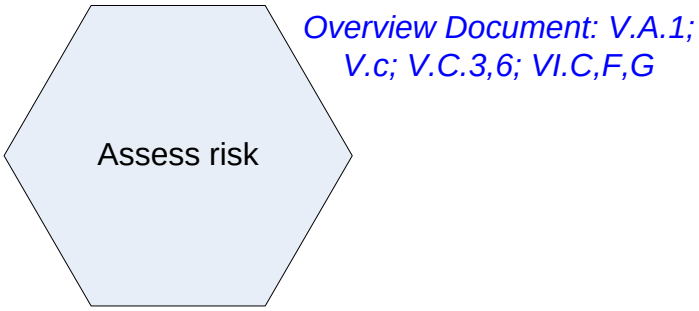
- Consider toxicity and exposure in light of the active ingredient and it formulations



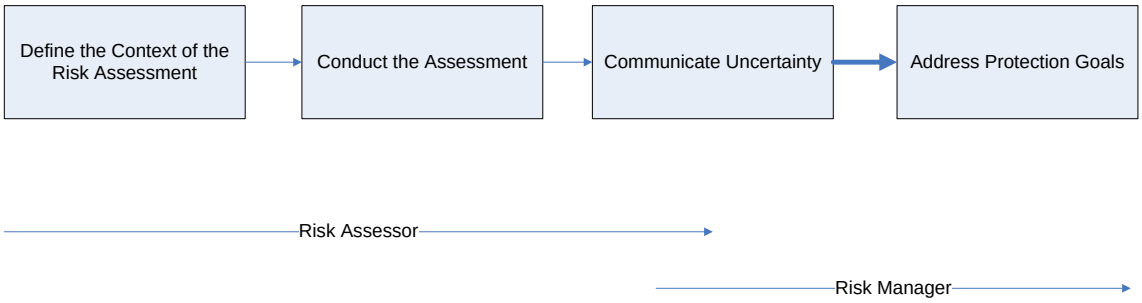
- Extrapolate toxicity to potentially affected taxonomic classes of organisms



- ***Fifth work module (Chapter 5)***



- Utilize best available data and methodologies to FIFRA and ESA



BIBLIOGRAPHY

Asfaw, A., M.R. Ellersieck, and F.L. Mayer. 2003. Interspecies correlation estimations (ICE) for acute toxicity to aquatic organisms and wildlife. II. User Manual and Software. EPA/600/R-03/106. U.S. Environmental Protection Agency, National health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. 14 p.

Asher, J.E. and D.W. Harman. 1995. Invasive exotic plants are destroying the naturalness of U.S. Wilderness areas. *International J. of Wilderness* 1(2):35-37

Beyers, D.W., T.J. Keefe, and C.A. Carlson. 1994. Toxicity of carbaryl and malathion to two federally endangered fishes, as estimated by regression and ANOVA. *Environmental Toxicology and Chemistry* 13:101-107.

Burns, L.A., D.M. Cline, and R.R. Lassiter. 1982. Exposure analysis modeling system (Exams): User manual and system documentation. EPA-600/3-82-023, U.S. Environmental Protection Agency, Athens, Georgia, USA.

Burns, L.A. 2000. Exposure analysis modeling system (EXAMS). Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Athens, GA.

Carpy, S.A., W. Kobel, J. Doe. 2000. Health risk of low-dose pesticides mixtures: A review of the 1985-1998 literature on combination toxicology and health risk assessment. *Journal of Toxicology and Environmental Health Part B: Critical Reviews* 3(1): 1-25.

Carsel, R.F., A.E. Smith, L.A. Mulkey, J.D. Dean, and P. Jowise. 1984. User's manual for the pesticide root zone model (PRZM): Release 1. U.S. Environmental Protection Agency, Athens, Georgia, USA.

Carsel, R.F., J.C. Imhoff, P.R. Hummel, J.M. Cheplick and J.S. Donigian, Jr. 1997. PRZM-3, A model for predicting pesticide and nitrogen fate in crop root and unsaturated soil zones: Users manual for Release 3.0. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Athens, GA.

Cassee, F.R. and J.P. Groten. 1998. Toxicological evaluation and risk assessment of chemical mixtures. *Crit Rev Toxicol*. 28(1):73-101.

Chapman, P.M., A. Fairbrother, and D. Brown. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology and Chemistry* 17:99-108.

Code of Federal Regulations Title 40, Part 160 (2002) Environmental Protection Agency Good Laboratory Practice Standards.

Council on Environmental Quality, Office of the President (CEQ, 1997). Considering cumulative effects under the national environmental policy act. [A 2000 version is available here: http://ceq.eh.doe.gov/nepa/ccenepa/ccenepa.htm]

Driver, C.J., M.W. Ligotke, P. VanVoris, B.D. McVeety, B.J. Greenspan, and D.B. Drown. 1991. Routes of uptake and their relative contribution to the toxicologic response of northern bobwhite *(Colinus virginianus)* to an organophosphate pesticide. *Environmental Toxicology and Chemistry* 10(1): 21-33.

Duke, L.D. and M. Taggart. 2000. Uncertainty factors in screening ecological risk assessment. *Environmental Toxicology and Chemistry* 19:1668-1680.

Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM). 1999. ECOFRAM Draft Terrestrial Report. May 10, 1999. [Also listed as "ECOFRAM draft Terrestrial Report, 1999, Technical Progress Report to the Scientific Advisory Panel Terrestrial Assessments 3/14/00"] http://www.epa.gov/oppefed1/ecorisk/terrreport.pdf

Fairchild, J.F., A. Allert, L.S. Sappington, K.J. Nelson, and J. Valle. 2008. Using accelerated life testing procedures to compare the relative sensitivity of rainbow trout and the federally listed threatened bull trout to three commonly used rangeland herbicides (picloram, 2,4-D, and clopyralid). *Environmental Toxicology and Chemistry* 27:623-630.

Fletcher, J.S., J.E. Nellessen, and T.G. Pfleeger. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, and instrument for estimating pesticide residues on plants. *Environmental Toxicology and Chemistry* 13:1383-1391.

Frawley, John P. 1957. Marked potentiation in mammalian toxicity from simultaneous administration of two anticholinesterase compounds. *Journal of Pharmacology and Experimental Therapeutics* 121(1): 96-106.

Giddings, J.M., P. Hendley, A.J. Hosmer, and K.H. Reinert. 2000. Extrapolation factors, levels of concern, and additional toxicity testing in the early stages of a pesticide risk assessment. Abstracts, Society of Environmental Toxicology and Chemistry 21st Annual Meeting, Nashville TN, November 12-16.

Green, R.E. 1998. Long-term decline in the thickness of eggshells of thrushes, *Turdus* spp., in Britain. *Proceedings of the Royal Society B.* 263(April 22):679-684.

Hoerger, F., and E.E. Kenaga. 1972. Pesticide residues on plants: Correlation of representative data as a basis for estimation of their magnitude in the environment. In

Environmental Quality and Safety: Chemistry, Toxicology, and Technology, F. Coulston and F. Kort (eds.), Georg Theime, Stuttgart, West Germany, pp. 9–28.

Huber, Don M., et al. 2002. Invasive pest species: Impacts on agricultural production, natural resources, and the environment. Council for Agricultural Science and Technology Issue Paper 20.

Jackson, Scott and David Gustafson. 2003. Methodology for characterizing exposure to surface and ground water from inert compounds. Authored for CropLife America, November 2003 (draft).

Leonard, R.A., W.G. Knisel and D.A. Still. 1989. GLEAMS : Groundwater loading effects of agricultural management systems. *Transactions American Society of Agricultural Engineering.* 30: 1403-1418.

Lincer, Jeffrey L. 1975. DDE-induced eggshell-thinning in the American kestrel: a comparison of the field situation and laboratory results. *Journal of Applied Ecology* 12:781-793.

Macek, K.J. and W.A. McAllister. 1970. Insecticide susceptibility of some common fish family representatives. *Transactions of the American Fisheries Society* 99:20-27.

Mayer, F.L. Jr. and M.R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and database for 410 chemicals and 66 species of freshwater animals. Resource Publication 160, U.S. Fish and Wildlife Service, Washington, DC.

McCarty, L.S. and C.J. Borgert. 2006. Review of the toxicity of chemical mixtures: Theory, policy, and regulatory practice. *Regulatory Toxicology and Pharmacology* 45(2): 119-143. May 15, 2006

McGaughey, Bernalyn D. 2005. Supporting the expert by arguing from information theory in *Scientific Evidence and the Expert Witness*, James Brown, Ed. Aspen Press.

Moore, A., and C.P. Waring. 1996. Sublethal effects of the pesticide diazinon on olfactory function in mature male Atlantic salmon parr. *Journal of Fish Biology* 48: 758- 775.

Mullin, Barbara H., L.W.J. Anderson, J.M. DiTomaso, R.E. Eplee, and K.D. Getsinger. 2000. Invasive Plant Species. Council for Agricultural Science and Technology, Issue Paper 13.

Pimentel, D., R. Zuniga, and D. Morrison. 2004. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273-288.

Powell, D.B. and R.C. Palm. 2007a. Effects of environmentally relevant levels of diazinon on the olfactory function and behavior of Chinook salmon in two-choice maze systems. Abstracts, Society of Environmental Toxicology and Chemistry Annual Meeting, Milwaukee, WI, November 2007.

Powell, D.B. and R.C. Palm. 2007b. Predator avoidance by juvenile Chinook salmon (*Oncorhynchus tshawytscha*) following exposure to sublethal levels of diazinon. Abstracts, Society of Environmental Toxicology and Chemistry Annual Meeting, Milwaukee, WI, November 2007.

Raimondo, S., P. Mineau, and M.G. Barron. 2007a. Estimation of chemical toxicity to wildlife species using interspecies correlation models. *Environmental Science and Technology* 41:5888-5894.

Raimondo, S., D.N. Vivian, and M.G. Barron. 2007b. Web-based interspecies correlation estimation (Web-ICE) for acute toxicity: User manual. Version 1.1. EPA/600/R-07/071. Gulf Breeze, FL.

Ratcliffe, D.A. 1967. Decrease in eggshell weight in certain birds of prey. *Nature* 215:208-210.

Raybould, Alan. 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environmental Safety Research* 5: 119-125.

Sappington, L.C., F.L. Mayer, F.J. Dwyer, D.R. Buckler, J.R. John, and M.R. Ellersieck. 2001. Contaminant sensitivity of threatened and endangered fishes compared to standard surrogate species. *Environmental Toxicology and Chemistry* 20:2869-2876.

Scholz, N.L., N.K. Truelove, B.L. French, B.A. Berejikian, T.P. Quinn, E. Casillas, and T.K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Science* 57:1911-1918.

USEPA. 1992. Framework for Ecological Risk Assessment. Risk Assessment Forum. EPA/630/R-92/001. February 1992

USEPA . 1993. Wildlife exposure factors handbook (Volumes I and II). EPA/600/R-93/187a. December 1993.

USEPA. 1998. Guidelines for Ecological Risk Assessment, p. 65 (as published in 63FR26846).

USEPA. 1998a. Registration Eligibility Decision (RED) – Triclopyr, p. 70. October 1998 EPA 738-R-98-011

USEPA. 2008. Risks of propyzamide use to federally threatened California red-legged frog (*Rana aurora draytonii*). Pesticide Effects Determination. February 7, 2008.

USEPA Office of Science Policy. 2000. Science policy council risk characterization handbook. EPA 100-B-00-002. December 2000. URL: http://www.epa.gov/osa/spc/pdfs/rchandbk.pdf

USEPA OPPTS (various dates) Harmonized test guidelines. A 10-series set of testing guidelines. To access guidelines see http://www.epa.gov/opptsfrs/home/guidelin.htm.

USEPA OPP. 1999. Memorandum, Morton to White: Trichlorfon (057901); List A Reregistration Case No. 0104; Product and Residue Chemistry Chapters for the Reregistration Eligibility Document (RED). DP Barcode D257225. June 24, 1999.

USEPA OPPTS OPP. 2004a. Overview of the ecological risk assessment process in the office of pesticide programs, U.S. Environmental Protection Agency: Endangered and threatened species effects determinations. January 23, 2004. URL: http://www.epa.gov/espp/consultation/ecorisk-overview.pdf

USEPA OPPTS OPP. 2004b. A discussion with the FIFRA Scientific Advisory Panel regarding the terrestrial and aquatic Level II refined risk assessment models (Version 2.0). Presented at the March 30 – April 2, 2004 FIFRA SAP Meeting and available at http://www.epa.gov/oppefed1/ecorisk/ecorisk_fifrasap.htm#Year2004

USEPA OPPTS OPP. 2006a. Technical overview of ecological risk assessment problem formulation. http://www.epa.gov/oppefed1/ecorisk_ders/toera_problem.htm (accessed on May 30, 2007)

USEPA OPPTS OPP. 2006b. Follow-up to September 28, 2006 Oversight Hearing: USFWS and US NMFS (Services, undated) Guidance for conducting literature searches for Section 7 consultation (undated draft)

US FWS and US NMFS. Services, 1998. Endangered species consultation handbook: Procedures for conducting consultation and conference activities under Section 7 of the Endangered Species Act. March 1998. URL: http://www.fws.gov/endangered/consultations/s7hndbk/s7hndbk.htm

US FWS and US NMFS. Services, 2003. Organizing our thinking for dealing with the suite of EPA Pesticide/ESA issues. Internal memo dated March 7, 2003

US FWS and US NMFS. Services, 2004. Services' evaluation of EPA's ecological risk assessment process. Letter to Susan B. Hazen, Principal Deputy Assistant Administrator, USEPA OPPTS, from Steve Williams, Director USFWS, and William T. Hogarth, Assistant Administrator, NMFS. January 26, 2004. URL: http://endangered.fws.gov/consultations/pesticides/evaluation.pdf

US FWS and US NMFS. Services, 2006. A framework to assist in making endangered species act determinations of effect for individual or grouped actions at the bull trout subpopulation watershed scale. In Rock Creek Mine Biological Opinion, Part B, Appendix G (October 2006)

US Geological Survey. 2006. Pesticides in the nation's streams and ground water, 1992-2001. USGS Circular 1291. March 3, 2006

US NOAA. Undated. Consultation initiation template and users guide. NOAA Fisheries Northwest Region. URL: http://www.cit.noaa.gov/nosign/

Warren-Hicks, William. 2007. Personal communication. May 30, 2007

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. *BioScience* 48: 607-615.

Willis, G.H., and L.L. McDowell. 1987. Pesticide persistence on foliage. *Rev. Environ. Contam. Toxicol*. 100:23–73.