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GUIDANCE FOR IDENTIFYING PESTICIDE CHEMICALS AND OTHER SUBSTANCES THAT HAVE A COMMON MECHANISM OF TOXICITY

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EXECUTIVE SUMMARY

The Food Quality Protection Act (FQPA) of 1996 requires the United States Environmental Protection Agency (EPA) to assess the cumulative risks to human health that can result from exposure to pesticides and other substances that are toxic by a common mechanism. The Agency is currently developing a process for performing cumulative risk assessments of this type. Such assessments will play an increasingly important role in the evaluation of risks posed by pesticides, and will improve the Agency's ability to make regulatory decisions that fully protect public health and sensitive subpopulations, including infants and children.

The identification of pesticides and other substances that cause a common toxic effect by a common mechanism is the first step of the cumulative risk assessment process. This document describes the approach that EPA will use for identifying pesticides and other substances that cause common toxic effects by common mechanisms of toxicity. Specifically, this document describes: EPA's interpretation of common mechanism of toxicity with respect to making a determination of safety; the specific steps that will be taken for identifying mechanisms of toxicity of pesticides and other substances that cause a common toxic effect; the types of data and their sources that are needed; how these data are to be used in reaching conclusions regarding commonality of mechanisms of toxicity; and criteria the Agency will use for categorizing pesticides and other substances for purposes of cumulative risk assessments. Details on the other aspects of the cumulative risk assessment process will be discussed in a separate document.

This document was developed from a draft version entitled *Guidance for Identifying Pesticide Chemicals that Have a Common Mechanism of Toxicity, for Use in Assessing the Cumulative Toxic Effects of Pesticides,* that was released for public comment in August of 1998 (FR 63 42031, FRL-5797-7). The Agency received comments from various organizations. Each of the commentors offered recommendations for improving the science policy. All comments were extensively evaluated and considered by the Agency. This revised version embodies many of the sentiments and recommendations of the commentors. The public comments, as well as a detailed summary of the Agency's response to the comments are being made available in the Federal Register.

I. INTRODUCTION

The Food Quality Protection Act (FQPA) of 1996 stipulates, among other things,[1] that when determining the safety of a pesticide chemical EPA shall base its assessment of the risk posed by the pesticide chemical on: aggregate (i.e., total dietary, residential, and other non-occupational) exposure to the pesticide and available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances[2] that have a common mechanism of toxicity. The Act specifically mandates the Agency to consider the special susceptibility of infants and children to the toxic effects caused by pesticides. The Agency must also base its risk assessment on available information concerning the *cumulative* effects on infants and children to the pesticide and other substances that have a *common mechanism of toxicity*. The reason for consideration of these factors is due to the possibility that low-level exposures to multiple substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the chemicals individually. A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Hence, in assessing the risks posed by a given pesticide chemical, EPA must assess the cumulative risks to human health that can result from exposure to the pesticide, as well as from other pesticide chemicals and other substances that are toxic by a common mechanism.

The goal of a cumulative risk assessment, in regard to implementing FFDCA as amended by FQPA, is to characterize the potential for a cumulative toxic effect and the magnitude of the effect in individuals exposed to pesticides and other substances that cause a common toxic effect by a common mechanism. In order to assess these cumulative toxic effects, the Agency needs to first identify and categorize those pesticides and other substances that cause a common toxic effect by a common mechanism. **The purpose of this document is to describe the approach that EPA will use for identifying and categorizing pesticides and other substances that cause common toxic effects from common mechanisms of toxicity.** Specifically, this document describes:

- EPA's interpretation of common mechanism of toxicity with respect to making a determination of safety under FFDCA as amended by FQPA;
- The specific steps that need to be taken for identifying mechanisms of toxicity of pesticides and other substances that cause a common toxic effect;
- The types of data (and their sources) that are needed for

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[1] For details see *The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) As Amended by the Food Quality Protection Act (FQPA) of August 3, 1996;* U.S. Environmental Protection Agency, Office of Pesticide Programs, document # 730L97001, March, 1997.

[2] "Other substances" includes pesticide chemicals, pharmaceutical substances (e.g., drug products), industrial chemicals, and other substances to which the general population is exposed.

doing so;

- How these data are to be used in reaching conclusions regarding commonality of mechanisms of toxicity;

- Criteria the Agency will use for categorizing pesticides and other substances for purposes of cumulative risk assessments.

This document does not address how EPA will assess cumulative toxicity when making determinations of safety. This topic will be discussed in a forthcoming Agency science policy document.

II. DEFINITIONS OF TERMS

This document uses a number of terms that are necessary for discussions of toxic effects, mechanism of toxicity, and the identification of substances that cause a common toxic effect by a common mechanism. These terms are not defined (some are not mentioned) in FQPA. The definitions presented here represent EPA's interpretation of the terms for purposes of implementing the requirements of FQPA.

Analog(s). Analog is a generic term used to describe substances that are chemically closely related. Structural analogs are substances that have similar or nearly identical molecular structures. Structural analogs may or may not have similar or identical biological properties.

Toxic Effect. A toxic effect is an effect known (or can reasonably be expected) to occur in humans, that results from exposure to a chemical substance and that will or can reasonably be expected to endanger or adversely affect quality of life. Some examples of toxic effects are acute lethality, loss of hearing, renal tubule necrosis and cardiomyopathy, to name just a few.[3]

Site of a Toxic Effect. The site of a toxic effect is the specific anatomical or physiological site or locus (e.g., organ or tissue) at which the effect occurs.

Common Toxic Effect. A pesticide and another substance that are known to cause the same toxic effect in or at the same anatomical or physiological site or locus (e.g., same organ or tissue) are said to cause a common toxic effect. Thus, a toxic effect observed in studies involving animals or humans exposed to a pesticide chemical is considered common with a toxic effect caused by another chemical if there is concordance with both site and nature of the effect.

Cumulative Toxic Effect. A cumulative toxic effect is the net change in magnitude of a common toxic effect resulting from exposure to two or more substances that cause the common toxic effect by a common mechanism, relative to the magnitude of the common

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[3] Toxic effect is not synonymous with toxic endpoint. Toxic endpoint is a quantitative expression of a toxic effect occuring at a given level of exposure. For example, acute lethality is a toxic effect, whereas an LD_{50} value (median lethal dose) is the toxic endpoint that pertains to the effect.

toxic effect caused by exposure to any of the substances individually.

Toxophore. Substances that are capable of causing a toxic effect contain a structural feature or moiety that bestows the toxic property. This structural feature or moiety is referred to generically as the toxophore, or toxophoric moiety[4]. A toxic substance elicits its toxicity through interaction of its toxophore with a biomolecular site (e.g., receptor)[5] in cells of tissue or organs to cause changes or alterations in normal cellular biochemistry. These biochemical changes or alterations lead to disruption of the physiological process(es) the tissue or organs perform and, ultimately, the toxic effect. The toxicity of many substances, however, is not due to a direct interaction with a biomolecular site. Rather, the toxicity results from metabolism of a structural substituent to a toxophore, which then causes the toxicity. Metabolic pathways that lead to toxicity are often called **bioactivation pathways.**

Mechanism of Toxicity.[6] Mechanism of toxicity is defined as the major steps leading to a toxic effect following interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction that are required in order to describe a mechanism of toxicity. Generally, the more that is understood about the various steps in the pathway leading to an adverse effect, the more confident one is about the mechanism of toxicity. For instance, a mechanism of toxicity may be described by knowing the cascade of effects such as the following: a chemical binds to a given biological target *in vitro,* and causes the receptor-related molecular response; *in vivo* it also leads to the molecular response and causes a number of intervening biological and morphological steps that result in an adverse effect. Other processes may describe a mechanism of toxicity in other cases.

Common Mechanism of Toxicity. Common mechanism of toxicity pertains to two or more pesticide chemicals or other substances that cause a common toxic effect to human health by the same, or essentially the same, sequence of major biochemical events. Hence, the underlying basis of the toxicity is the same, or essentially the same, for each chemical.

Toxic Action. Toxic action of a given substance is its interaction with biological targets, to lead to a toxic effect.

Site of Toxic Action. The site of toxic action of a given substance is the anatomical or physiological site(s), locus, or loci at which takes place the interaction of the substance with its biological targets, to lead to a toxic effect.

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[4] The term "toxophore" with respect to toxic substances, is akin to the term "pharmacophore" with respect to drug substances: the pharmacophore is that structural moiety of a drug substance or substances which imparts a desired pharmacological property.

[5] A biomolecular site refers to a specific area on a particular type of biomolecule (e.g, DNA, RNA, peptide, protein, lipoprotein, enzyme, etc.) within a cell. The toxophoric portion of a given pesticide may interact reversibly or irreversibly with its biomolecular site, depending upon the reactive nature of the toxophore and the biomolecular site.

[6] In the context of this document, mechanism of toxicity refers to the mechanism by which a pesticide substance is toxic to humans or experimental animals, and not the mechanism by which it is toxic to target or intended species (i.e., its mechanism of pesticidal action). With some pesticides, however, the mechanism responsible for causing toxicity to humans or experimental animals is similar to the mechanism of pesticidal action.

Structure-Activity Relationships. Substances that contain or are bioactivated to the same toxophore may cause a common toxic effect by a common mechanism. The relative toxic efficacy and potency[7] among the substances in their ability to cause the toxic effect may vary. Differences in potency or efficacy are directly related to: the specific or incremental structural differences between the substances; the influence these differences have on the ability of the toxophore to reach and interact with its biomolecular site of action; and on the intrinsic abilities of each of the substances to cause the effect. The ability of two or more structurally-related substances to cause a common toxic effect and the influence that their structural differences have on toxic efficacy and potency are referred to as structure-activity[8] relationships.

Weight-of-Evidence. Weight-of-evidence refers to a qualitative scientific evaluation of a chemical substance for a specific purpose. A weight-of-evidence evaluation involves a detailed analysis of several or more data elements, such as data from different toxicity tests, pharmacokinetic data, and chemistry data, followed by a conclusion in which a hypothesis is developed, or selected from previous hypotheses.

III. Process for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity.

To assess the cumulative toxicity of pesticides and other substances that cause common toxic effects by common mechanisms, EPA will first need to identify those pesticides and other substances that cause common toxic effects by common mechanisms, and then group them in accordance with commonality of toxic effect and toxic mechanism. Once grouped, combined risk assessments can be performed and the potential for cumulative toxicity that may result from exposure to substances within a group can be characterized.

The conceptual framework of the process that EPA will use to identify pesticide chemicals and other substances that cause a common toxic effect by a common mechanism is illustrated in Figure 1. This process is designed to enable EPA to make accurate identification and categorization of pesticides and other substances that are toxic from a common mechanism, in both a timely and resource-effective manner. (Specific examples of the application of this process are in preparation, and will be made available at a future date.) To implement the process, the Agency has convened a multidisciplinary team of EPA scientists who are experts in chemistry, biology, pharmacology, toxicology and pharmacokinetics. It is the responsibility of this team to identify and analyze data and information pertaining to toxic mechanisms, and to make expert judgements regarding mechanisms of toxicity of pesticides and other substances. The following policies and practices will be used by the Agency for identifying chemicals that have a common mechanism of toxicity:

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[7] *Toxic efficacy* is the intrinsic ability for a substance to produce a given toxic effect. *Maximal toxic efficacy* is reached when an increase in dose no longer causes an increase in the magnitude (intensity) of the effect. *Toxic potency* is the magnitude of the toxic effect that results from a given exposure level (or dose), or the range in magnitude of the toxic effect that corresponds to a range in levels of exposure. *Relative toxic potency* refers to a comparison of the exposure level or dose required of an individual substance to the exposure levels or doses required of other substances to cause a common toxic effect of an equivalent magnitude (e.g, LD_{50}, ED_{50}) by a common mechanism of toxicity.

[8] In the context of this document the term "activity" is synonymous with toxicity.

- A thorough identification and analysis of all relevant information will be undertaken for each pesticide chemical and other substances under consideration. This will provide the basis for identifying underlying mechanisms of toxicity;

- A "weight-of-evidence" approach will be used to support the development of hypotheses pertaining to mechanisms of toxicity. Generally, no single piece of information will suffice to support the

- External review of EPA's decisions concerning: utilization of established toxic mechanisms; determination of toxic mechanisms for specific substances; and grouping of substances by mechanism of toxicity will be solicited as needed .

When identifying toxic effects, common toxic effects, mechanisms of toxicity, and common mechanisms of toxicity for purposes of grouping substances that cause a common toxic effect by a common mechanism of toxicity, care must be taken not to confuse "mechanism of toxicity" with "site of toxic action," or "site of toxic action" with "toxic effect" or "site of toxic effect." (These terms are defined near the beginning of this document.) With many substances, the site of a toxic effect is the same as the site of toxic action. It is also true, however, that with many other substances the site of a toxic effect may be different than site of toxic action. For example, a substance inhibits the catalytic activity of the peroxidase enzyme within the thyroid gland. Inhibition of this enzyme prevents the synthesis of thyroxine and triiodothyronine, and ultimately leads to hypothyroidism, the toxic effect. In this case, the site of the toxic effect is the same as the site of toxic action: the thyroid gland. Another substance known to cause hypothyroidism does so by preventing the synthesis of thyroid-stimulating hormone within the anterior pituitary gland. Here the site of the toxic effect is the thyroid gland, but the site of toxic action is the anterior pituitary gland. Although these two substances cause a common toxic effect, they would not be considered for cumulative risk assessment because they have different mechanisms of toxicity.

Many substances can cause more than one toxic effect, depending upon level of exposure, and do so by different mechanisms of toxicity that take place at different sites of toxic action. However, a chemical may also cause multiple toxic effects at multiple sites from a single mechanism of toxicity taking place at a single site of toxic action, provided that the function initially altered at the site of toxic action normally controls other functions at distant sites. For example, a substance that prevents the conversion of cholesterol to corticosteroid hormones in the adrenal cortex would ultimately cause many effects throughout the body that would differ in site and nature. The Agency will group substances that cause multiple toxic effects by a common mechanism from a common site of toxic action (e.g., the multiple effects caused by certain endocrine disruptors) for purposes of cumulative risk assessment, provided at least one of the toxic effects is common among the substances.

Step 1. Identify a Candidate Set of Substances That Might Cause a Common Toxic Effect by a Common Mechanism of Toxicity. The process of identifying pesticides and other substances that have a common mechanism of toxicity begins with a preliminary grouping of chemicals that might cause a common toxic effect by a common mechanism of toxicity (step 1, Figure 1). Substances that are related structurally, or have a similar mechanism of pesticidal action, or share a general mechanism of mammalian toxicity or cause what could be a common toxic effect in humans or experimental animals are those that could cause a common toxic effect by a common mechanism. Hence, the initial, preliminary grouping of substances will be based upon at least one of the following criteria:

- structural similarity;
- mechanism of pesticidal action;
- general mechanism of mammalian toxicity;
- a particular toxic effect.

Use of structural similarity as a starting point for grouping chemicals relies on the assumption that substances that are structurally analogous could contain a common toxophore (or may yield a common toxophore upon metabolism) and may interact analogously with cellular biomolecular sites to cause a common toxic effect. To identify pesticides and other substances that are structurally similar, the Agency will perform substructure searches in databases containing: registered pesticides; pesticides for which there are import tolerances; and other substances (e.g., pharmaceuticals, industrial chemicals) that are used in commerce in the United States. Search queries for identification of structurally similar substances may include, for example: toxophore (if known) or metabolic precursor of the toxophore; base structure; and accompanying functional groups or other substituents that may impact on the propensity of a substance to produce a toxicological response common with those of structurally-related chemicals.

Preliminary grouping of pesticides based on mechanism of pesticidal action is justifiable because the mechanisms by which a number of pesticides are toxic to humans are fundamentally similar or, in some cases, identical to their mechanisms of intended toxicity to pests. With such pesticides the portion of the molecule that is responsible for pesticidal action is also responsible for human toxicity (i.e., the portion of the molecule that bestows pesticidal activity is also the human toxophore). The pesticidal action and human toxicity of these pesticides are often due to analogous interactions of their toxophores with specific biomolecular sites that are common to pests and humans, respectively.

Preliminary grouping of pesticides and other substances that share a general mechanism of mammalian toxicity is based on the possibility that such substances may cause a common toxic effect. Examples of general mechanism of toxicity include, for example, substances that uncouple oxidative phosphorylation, or substances that are known to undergo the same or similar bioactivation pathways, or that are metabolized to the same or analogous metabolites that are toxic.

Preliminary grouping of pesticides and other substances that cause a particular toxic effect known to occur in experimental animals or humans is based on the possibilty that the effect could be common (i.e., concordant in both site and nature), and that commonality in toxicity among two or more substances could be due to a common mechanism. Since this type of grouping is functionally-based, not structure-based, it enables the identification of structurally unrelated substances that cause a common toxic effect from a common mechanism that otherwise may not be identifiable from groupings based on structural similarity or mode of pesticidal action alone.

Not all toxic effects can be used as a preliminary basis for grouping substances. Toxic effects which have many possible unrelated causes, or which could be defined as nonspecific in origin are not appropriate as the primary basis for initial grouping of chemicals. These effects, such as body weight changes or death, can result from many unrelated factors and are usually of limited value in understanding mechanism of toxicity. Therefore, such generalized effects, which could have many different causes, ordinarily will not be used as a basis for initial grouping of pesticides. An exception, however, is genetic alterations. While genetic alterations can result from a variety of causes, knowledge of the mechanism by which a chemical substance causes genetic alterations can provide insight into the mechanism by which it causes adverse human health effects. Therefore, data for chemicals with common mutagenic effects may serve as a basis for initial grouping of such chemicals.

Following preliminary grouping of substances using any of the criteria described above, other substances that are mammalian metabolic precursors to the substances identified under step 1 will be added to the initial grouping. The basis for including a metabolic precursor to a substance identified under step 1 is that since it is metabolized to the substance, it may cause a common toxic effect by a mechanism common with that of the substance.

It is important to emphasize and to make clear that the purpose of step 1 is for *preliminary grouping only*, and that substances (including any metabolic precursors) identified under this step will not be included in a cumulative risk assessment if it is determined that they do not cause a common toxic effect by a common mechanism. For example, while some substances that contain the same toxophore, or that are otherwise structurally analogous, may cause a common toxic effect by a common mechanism, others may not. It is also possible for substances to cause a particular toxic effect in which the nature of the toxic effect caused by each substance is the same, but the organ or specific location in the body where the effect occurs differs among the substances. It is also possible for two substances, even those that are structurally analogous, to cause entirely different toxic effects. Such differences between location or nature of a toxic effect can be ascribed to the specific structural and physicochemical differences between the substances, and the effect these differences have on their respective pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion of each substance) or pharmacodynamics (i.e., the interaction of the toxophore with biomolecular sites). In these instances the policy of the Agency is *not* to group such substances for cumulative risk assessment purposes, because the toxic effects are not common, as defined earlier. It is also possible with substances that cause a common toxic effect to cause the effect by different mechanisms of toxicity. Conversely, substances that share a general mechanism of toxicity may not necessarily cause a common toxic effect(s). Furthermore, substances that have a common mechanism of pesticidal action may not necessarily have a common mechanism of toxicity in mammals. Again, in these instances the policy of the Agency is not to group such substances for cumulative risk assessment purposes because they are not consistent with the definition of common mechanism of toxicity.

It must also be stressed that step 1 (like step 2 discussed below) is a very inclusive and necessary screening step to identify preliminary groupings of substances for a rigorous assessment. In particular, the criteria used in step 1 are very broad, and thus there is a real possibility that a substantial portion of the pesticide chemicals and other substances which are included in a preliminary grouping may not have a common mechanism of toxicity, and many will be dropped from a group in subsequent steps. Accordingly, EPA does not regard information which shows substances meet the step 1 (and step 2) criteria for grouping as reliable by itself to conclude that such substances have a common mechanism of toxicity. Nor does such information create a sufficient presumption of the existence of a common mechanism of toxicity that it compels EPA to complete the remaining steps described below before making its safety determination.

Hence, only those substances that EPA determines, through the in-depth review described below, cause a common toxic effect by a common mechanism will be considered for cumulative risk assessment. As shown in the Figure, this examination will involve: a thorough evaluation of toxicity data to determine which substances identified under step 1 cause a common toxic effect; determination of the mechanism of toxicity by which each substance causes the common toxic effect; and subsequent comparison of each mechanism to confirm or rule-out commonality. It is likely that EPA will conclude a substantial portion of the substances identified in step 1 should not be included in a cumulative risk assessment.

Step 2. Definitively Identify Those Substances from Step 1 That Cause a Common Effect. The primary purpose of step 2 is to further refine the preliminary grouping created at step 1 by screening out substances that obviously do not cause a *common toxic effect.* Following the preliminary grouping of substances (step 1, Figure 1) a detailed evaluation of available toxicology data for each substance will be undertaken to identify and characterize the toxic effects caused by each substance, and to determine which of the substances cause toxic effects that are common with other substances (i.e., toxic effects that are concordant in both site and nature). A primary data set to be used by EPA will be toxicity data generated in support of regulatory activities as outlined in 40 CFR 158. The Agency may also use toxicity data obtained from other studies, such as those described in government reports, or the published literature. The evaluation of toxicology data for purposes of identifying and characterizing toxic effects will be conducted in a manner similar to that used by EPA in its pesticide registration and re-registration programs.

Most substances, depending upon level of exposure, can elicit more than one toxic effect (albeit one toxic effect is generally more readily elicitable than the others). All toxic effects caused by each substance, regardless of the exposure levels required to induce the effects, will be evaluated and compared to the toxic effects caused by the other substances. Only those substances that cause a common toxic effect will remain grouped. Thus, for those substances initially grouped (step 1) using the "particular toxic effect" criterion (step 1), a determination as to which substances the toxic effect is in fact common will need to be made. The toxicity data for substances initially grouped using any of the other criteria in step 1 will also be evaluated to determine which of these substances cause a common toxic effect. Substances may be placed in more than one group in instances where substances cause more than one common toxic effect. Pesticide chemicals that do not cause a toxic effect that is common with at least one other substance identified under step 1 will be eliminated from the group and, thus, will not undergo further cumulative risk consideration.

Step 3. Determine the Toxic Mechanism(s) by Which Each Substance Causes a Common Toxic Effect. The next phase of the review process (step 3, Figure 1) is to determine the mechanisms by which the substances cause the common toxic effect(s) identified under step 2 (Figure 1). Generally, the more that is understood about the various biochemical events that lead to a toxic effect, the more apparent and scientifically acceptable is the mechanism of toxicity. While desirable, all of the specific biochemical events involving a substance in the causation of its toxicity do not need to be known or completely characterized in order to describe its mechanism of toxicity. What is needed, as a minimum, is an understanding of those biochemical events that are most crucial in causing the toxicity. Once the critical biochemical events pertaining to toxicity are understood for each substance, they can be compared and identification of those substances that are toxic from a common mechanism can be made. Hence, the goal of step 3 is to determine, to the extent possible for each substance identified under step 2 as causing a common toxic effect, those biochemical events that are most critical in causing the effect.

The toxic mechanisms of some classes of substances in causing a given toxic effect have been characterized, and are described in various literature sources (e.g., textbooks, journals, etc.). These mechanisms were elucidated from the development and comprehensive analysis of data pertaining to the structure, pharmacokinetics and toxicity of the substances and their analogs. The toxophoric moieties and structure-activity relationships of many of these chemical classes were similarly characterized. The toxic mechanisms, toxophores and structure-activity relationships of other pesticides, however, have not been fully characterized, either because of insufficient data, or because available data have not yet been fully analyzed.

Rather than reexamining *de novo* all of the relevant data, EPA will assume that a substance is toxic by the mechanism that has been previously determined provided that the mechanism is consistent with current toxicological theory and deemed scientifically plausible by the Agency for these purposes. Thus, identification of toxic mechanisms will involve an initial search of Agency databases and the literature (step 3a, Figure 1) for assessments or studies that describe mechanisms of toxicity for any of the pesticides grouped in step 2. The types of literature sources that will be searched and used include

standard reference and text books, peer-reviewed journals, government reports, and study reports submitted to the Agency. This will allow segregation of the substances into two sub-groups: those for which mechanism(s) of causing a common toxic effect have been determined; and those for which their mechanism(s) have not been determined. When deemed necessary, more comprehensive literature or Agency database searches will need to be conducted to identify data that support or invalidate previously determined mechanisms of toxicity for which uncertainty exists.

EPA will attempt to determine the mechanisms of toxicity of those substances whose toxic mechanisms are not known or not well understood, or for which there is an absence of direct mechanistic data. The determination of a toxic mechanism will be based upon an evaluation of various data elements. The types of data and information that the Agency will use to develop a scientifically defensible determination of a given pesticide's toxic mechanism are structural data, pharmacokinetic data, and toxicity data. In situations in which such data are not available or are insufficient for a pesticide, the Agency will review and may use mechanistic, structural, pharmacokinetic or toxicity data pertaining to one or more analogs of the pesticide (or other substance) as a basis for determining the toxic mechanism of the pesticide. Identifying and obtaining pesticide or analog data will involve a comprehensive search of the literature and Agency databases. A primary source of these data and information will be studies that have been submitted to the Agency in support of registration and reregistration decisions. Other sources of data will include peer-reviewed journals, text books and government reports.

The Agency will analyze these data and, using a weight-of-evidence approach, will attempt to determine the major biochemical events involving a pesticide (or other substance) that are most critical in causing its toxicity (step 3b). From an analysis of a substance's structure, for example, the recognition of moieties that are known or expected to react with biological macromolecules, or are known or expected to be metabolized to reactive (e.g., radical, electrophilic) intermediates, or are otherwise known or expected to bestow toxicity may allow one to infer one or more biochemical events that are responsible for the substance's toxicity. Data that define the metabolism, distribution and excretion of a pesticide in the body are also very useful for determining its mechanism of toxicity. Metabolism data that show the formation of toxic metabolites *in vivo* are especially useful for characterizing metabolic pathways which may be operative in causing toxic effects. Distribution and excretion data show the partitioning patterns of a substance in the body, and may in some cases be used to infer the types of metabolic transformations that are most likely to occur and where they are most likely to take place. These data, in conjunction with structural and toxicity data, may also provide explanations for differences in toxicity of structurally similar substances. Toxicity data can be helpful in the determination of a toxic mechanism in many ways. Genetic alterations, for example, are important in the causation of cancers and developmental effects. Tests for genetic alterations that show that a substance (or a metabolite thereof) forms a covalent adduct with DNA may be useful to infer or support a mechanism by which a pesticide known to cause cancer or developmental toxicity causes either of these effects.

Data pertaining to analogs of a pesticide or other substance will be reviewed and may be used in situations in which mechanism-related data are not available for the pesticide. An established mechanism of toxicity of a pesticide's analog(s), for example, may serve as a basis for determining the toxic mechanism of the pesticide. Conclusions based on the toxic mechanisms of an analog or analogs will only be made when: there is evidence that shows that the toxicological effects caused by the pesticide and the analogs are common; there is sufficient evidence that supports the toxic mechanism of the analog(s); and there is sufficient evidence for the Agency to conclude that the mechanism of toxicity of the pesticide is common with the mechanism of toxicity of the analog(s). Pharmacokinetic, toxicity and structure-activity relationship data that are available for analogs of a pesticide will also be used as a basis for determining the toxic mechanism of the pesticide (step 3b, Figure 1). For example, it is stated above that test data pertaining to genetic alterations may be useful to infer or support a mechanism of a pesticide known to cause cancer or developmental toxicity. Genetic alterations data available for analogs of a pesticide known to cause cancer or developmental toxicity may be useful for inferring the mechanism by which the pesticide causes these effects. Genetic alterations that are similar among a pesticide and its analogs are also useful in a

weight-of-evidence confirmation of the validity of such inferences, particularly when mechanistic data are available for the analog but not for the subject pesticide.

Relationships between structure and toxicity within a given series of structurally-similar substances or of a single given substance are often discernable from an analysis of: the general structure; the chemical properties of the substance(s); information pertaining to the pharmacokinetics and toxicity of the substance(s); and the structural differences within the series and their corresponding affect on toxic efficacy and potency. While knowledge of the mechanism of toxicity is usually not necessary in order to discern a causal relationship between structure and activity (toxicity), the relationship becomes more apparent and more useful when the mechanism of toxicity is known. Once deduced, the structure-activity relationship of the substance or the series can be useful for inferring the likelihood of an analogous, untested chemical to cause the same toxicological effect, and for estimating its toxic potency. In cases where the mechanism of toxicity is known for a substance or a group of substances, structure-activity relationships are useful for inferring the mechanism of toxicity of an analogous, untested substance and for supporting or refuting proposed mechanisms of toxicity of analogous untested substances.

Steps 4 and 5. Comparison of Mechanisms of Toxicity (Step 4) and Refined Grouping of Substances (Step 5). Once the mechanism of toxicity of each substance has been identified, comparisons of mechanisms will be made to determine which substances identified under step 2 as causing a given common toxic effect do so by a common mechanism. Determinations that two or more substances are toxic by a common mechanism will be based on similarities in both the nature and sequence of the major biochemical events that cause toxicity. Mechanistic similarities that would support a finding of a common toxic mechanism include, for example, analogous interactions of the pesticides or other substances with identical or similar biological targets, or the occurence of similar metabolic transformations that yield common or structurally analogous metabolites that interact with similar biological targets, or that are otherwise involved in causing toxicity. Substances that cause a common toxic effect by different mechanisms will excluded from the refined grouping (Step 5).

Peer review of EPA's decisions concerning: utilization of established toxic mechanisms; identification of toxic mechanisms for specific substances; and grouping (or non-grouping) of substances for purposes of cumulative risk assessment will be solicited in situations in which the Agency believes additional evaluation is needed to ensure that Agency decisions are consistent, well-reasoned and reflect current scientific thinking.

IV. Assessing the Cumulative Toxicity Posed by Two or More Substances That Are Toxic by a Common Mechanism.

Cumulative toxicity represents the net change in toxicity resulting from exposure to two or more chemical substances, relative to the toxicity caused by each substance alone. The evaluation of cumulative toxicity will be conducted in accordance to a cumulative risk assessment process being developed by the Agency. The goal of the cumulative risk assessment process, in regard to implementing FFDCA as amended by FQPA, is to characterize the potential for a cumulative toxic effect and the magnitude of the effect in individuals following known or anticipated exposures to substances that cause the effect by a common mechanism. Pesticide chemicals and other substances within a refined common mechanism grouping (step 5, Figure 1) will undergo cumulative risk assessment to determine the potential cumulative toxicity posed by exposures to such substances. This will involve consideration of a number of factors that pertain to: exposure; the pharmacokinetics of each substance; the nature of the common toxic effect; the pharmacodynamics of each substance in causing the effect;

pharmacokinetic or pharmacodynamic interactions that may take place between the substances; subpopulations for which exposures are anticipated; and susceptibility and sensitivity of exposed individuals or subpopulations to the common toxic effect.

A discussion of how these factors affect cumulative toxicity is beyond the scope of this document. In addition to the substances wihitn a refined grouping, the Agency will also consider the potential contribution to cumulative toxicity from other substances that undergo environmental degradation or metabolism in plants to any of the substances within the refined group. Substances that degrade in the environment, or that are metabolized in plants to substances in the refined grouping will be included in a cumulative risk assessment because such precursor substances may represent an additional source of exposure to the substances in the refined grouping. The cumulative risk assessment process that the Agency will use will be described in a forthcoming Agency science policy guidance document. The document will include detailed discussions of the above factors, how these and other factors will be considered by the Agency in assessing cumulative toxicity, and what the Agency will do when there are data gaps with the above factors.