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Selection of field and laboratory studies for environmental assessment*

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Abstract

Selection and conduct of the proper field and/or laboratory studies for inclusion in an Environmental Assessment of EPA and FDA regulated chemicals are often poorly understood or appreciated. This may lead to performance of inappropriate, invalid, or insufficient studies resulting in increased regulatory review time or rejection of submissions. The focus of this presentation is on studies listed in FDA and EPA guidelines. A rationale for environmental fate and effects testing is presented together with a tabulated summary of suggested tests by FDA and EPA for regulated chemicals. A description of studies, their significance, and a logical, efficient approach to selecting appropriate studies is also presented.

1. Introduction

The National Environmental Policy Act (NEPA) of 1969 is the federal regulation on which current environmental programs in the United States are based. This act mandates regulations to ensure a cleaner and healthier environment and requires that federal decisionmaking include an objective consideration of environmental impacts expected from proposed actions (21 CFR, Parts 25.1 to 25.5 and 40 CFR Parts 1500 to 1508, 1970). An Environmental Assessment (EA) is usually prepared by the manufacturer in advance of implementation of any direct federal actions such as approval of registration applications for chemicals, pesticides, human or animal health drugs. The EA

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documentation for all regulated chemicals is developed in compliance with NEPA regulations.

The U.S. Environmental Protection Agency (EPA) has use of several regulations under NEPA mandate that are designed to ensure that the development, testing, manufacture, use and disposal of chemicals does not cause adverse environmental or human health effects. The Toxic Substances Control Act (TSCA) regulates the use of new and existing chemical substances. Submission of a Pre-Manufacture Notification (PMN) and the Significant New Use applications are mandated under this regulation. The fate and effects testing program under TSCA is designed to evaluate the potential impact of manufactured chemicals released into the environment during manufacture, distribution and use.

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) regulates the manufacture, distribution and use of weedicides, insecticides, fungicides, and rodenticides as well as biochemicals (such as plant growth regulators) and biopesticides. The environmental fate and effects data under FIFRA are required to help assess (i) direct consequences through exposure to pesticides during application, or to pesticide residues remaining after application; (ii) indirect consequences from the persistence of widely distributed and persistent pesticide residues in the environment which may result in loss of usable land, water, and wildlife resources; and (iii) potential environmental exposure of other nontarget organisms such as fish and wildlife, endangered species or other populations at risk. The classes of chemicals regulated under FIFRA are not regulated under TSCA, and *vice versa*, unless the use patterns demand coverage under both.

The Clean Air Act, regulating the release of pollutants to the air, the Clean Water Act, regulating the release of pollutants to the navigable waters, the Resource Conservation and Recovery Act (RCRA), regulating the generation, treatment, storage and disposal of solid and hazardous wastes, and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) regulating cleanup of past hazardous waste release sites are also implemented by EPA and may require preparation of an EA.

Chemical entities regulated by the U.S. Food and Drug Administration (FDA) are also governed under NEPA. A major provision of NEPA is the requirement that applications to FDA for approval of a chemical entity, such as a new human or animal drug, must include sufficient environmental information to allow the agency to assess the environmental impact of manufacture, use and disposal of the chemical.

A variety of tests has been designed to help evaluate the potential fate and effects of a chemical in the environment. The objective of this paper is to describe such tests and the supporting rationale, their relative importance, and present a logical and efficient approach to selecting studies to support preparation of an EA. The discussion will be limited to chemicals regulated by EPA (TSCA, FIFRA) or FDA.

2. Environmental fate and effects testing required by EPA and FDA

2.1 Background information

The basic objective of an EA is to evaluate the potential environmental impacts due to the environmental introduction of a chemical substance. The environmental concentrations of a chemical are governed generally by its fate in the environment which is largely determined by the amounts of production, use and disposal patterns, its physical and chemical properties, mobility, chemical transformation, and biological degradation. Some basic differences in the environmental exposure patterns between EPA and FDA regulated chemicals are discussed below to help determine the degree of testing required under scenarios of different environmental exposure.

Fate and effects testing should be conducted in compliance with the Good Laboratory Practice (GLP) standards of EPA (40 CFR Part 160; EPA, 1990) or FDA (40 CFR Part 58; FDA, 1987). Submission of data that are not in compliance with GLP may lead to rejection of applications by the regulatory agencies. Exceptions to portions of data collected under non-GLP compliance may be sought from implementing regulatory agencies for a particular product.

Information on the production, use and disposal of regulated chemicals is essential in evaluating the Maximum Emitted Environmental Concentrations (MEECs) and also for the rational design of a fate and effects testing program. The primary use and geographical patterns of use and disposal vary from chemical to chemical. The use pattern of a chemical will determine the potential for its broader or narrower spectrum of environmental release. Agricultural chemicals and those used in aquaculture, by virtue of their applications to crops and water, and consequent direct releases to the environment, have much greater potential to enter into environmental compartments easily and rapidly than human drugs or chemicals used as raw materials in manufacturing processes. Pesticide movement is also governed by the interrelationships between the chemical and physical properties of the pesticide as well as soil, temperature, and rainfall in the region. Corn and soybean pesticides for example may have a potential for greater MEECs in the corn and soybean belt because of the large quantities used.

MEECs also depend on continuous or seasonal use and release to the environment. Human drugs may be used throughout the year and primarily enter the environmental compartments through domestic sewage, i.e. processing in Waste Water Treatment Plants (WWTP), and eventually through waste water discharge, i.e. the use and disposal of WWTP digested sludge. The use of agricultural chemicals may be seasonal. The use of chemicals for manufacturing processes, e.g, synthetic fibers, even though continuous, may have lesser potential for environmental release because the use of such chemicals is generally within a contained manufacturing facility. All uses, both minor and major, the forms of production and use (liquid or solid) and the disposal mechanisms should be taken into consideration in determining the MEECs.

TABLE 1

Environmental fate tests suggested by FDA for human and animal health drugs and other FDA regulated chemicals[a]

Guideline No.	Test
3.01	Water solubility
3.02	n-Octanol–water partition coefficient
3.03	Vapor pressure
3.04	Dissociation constant
3.05	Ultraviolet–Visible absorption spectrum
3.06	Melting temperature
3.07	Density and relative density
3.08	Sorption and desorption
3.09	Hydrolysis
3.10	Photodegradation
3.11	Aerobic biodegradation in water
3.12	Aerobic biodegradation in soil

[a] 21 CFR, Part 25, Federal Register, April 26, 1985; and Environmental Assessment Technical Assistance Handbook, March, 1987.

Generally, fate and effects testing is conducted on the active ingredient. In some cases formulated mixtures or mixtures of more than one active ingredient require characterizations of the active ingredient(s) as well as inert ingredients used for formulation. The assumption here is that even though the inert ingredients used in formulation may be inert with respect to the intended use of the chemical, the environmental impacts of the inert ingredient may be significant. A reading on whether to use active ingredient(s), or formulated mixtures, or formulations separate from the active ingredient should be obtained from the concerned regulatory agency before embarking on large scale fate and effects testing. The environmental fate and effects tests for EPA (TSCA, FIFRA), and FDA regulated chemicals are presented in Tables 1–6. As can be seen from these tables, there is a significant similarity for fate and effects testing required by these regulatory agencies. The differences are in the degree of testing, as well as some additional field testing required for pesticides.

It is important to realize that an appropriate testing plan depends upon the physical and chemical characteristics of each compound, how and where the compound enters the environment, and quantity of the compound entering the environment, i.e. a fixed set of required tests is not prescribed for all chemicals. It is recommended, however, that standard test procedures and guidelines be used in the conduct of environmental testing.

To assist in test standardization and thereby, data interpretation, the environmental staffs of the Center for Veterinary Medicine and the Center for Food Safety and Applied Nutrition have prepared an Environmental Assessment

TABLE 2

Environmental fate tests suggested by EPA under FIFRA[a]

Series No.	Test
Series 161: *Degradation studies*	
161-1	Hydrolysis studies
161-2	Photodegradation studies in water
161-3	Photodegradation studies in soil
161-4	Data for photodegradation studies in air
Series 162: *Metabolism studies*	
162-1	Aerobic soil metabolism studies
162-2	Anaerobic soil metabolism studies
162-3	Anaerobic aquatic metabolism studies
162-4	Aerobic aquatic metabolism studies
Series 163: *Mobility studies*	
163-1	Leaching and adsorption/desorption studies
163-2	Laboratory volatility studies
163-3	Field volatility studies
Series 164: *Dissipation studies*	
164-1	Field dissipation studies for terrestrial uses
164-2	Field dissipation studies for aquatic uses and aquatic impact uses
164-3	Dissipation studies for forestry uses
164-4	Dissipation studies for combination products and tank mix uses
164-5	Long term soil dissipation studies
Series 165: *Accumulation studies*	
165-1	Confined accumulation studies on rotational crops
165-2	Field accumulation studies on rotational crops
165-3	Accumulation studies on irrigated crops
165-4	Laboratory studies of pesticide accumulation in fish
165-5	Field accumulation studies of aquatic non-target organism

[a] 40 CFR, Part 158 — Subdivision N, 1982.

Technical Assistance Handbook (TAH) [1]. This handbook provides a valuable centralized source of information to persons gathering environmental data and preparing EAs. In addition, a number of Technical Assistance Documents are included which provide useful guidance in the preparation and conduct of these studies. Similar guidelines have been prepared by the staff of EPA, for FIFRA [3, 4], and TSCA [5, 6] regulated chemicals. It is recommended that the appropriate document be consulted for guidance before a testing plan is developed.

2.2 Physical and chemical properties

These properties of a chemical generally are useful for a preliminary evaluation of the potential fate, i.e. mobility and accumulation, of the chemical in the environment. The physical and chemical properties testing should therefore, take precedence.

TABLE 3

Environmental fate tests suggested by EPA under the Toxic Substances Control Act[a]

Section No.	Subpart B: Physical and Chemical Properties Test
796.1050	Absorption in aqueous solution: Ultraviolet/visible spectra
796.1220	Boiling point/boiling range
796.1370	Dissociation constants in water
796.1520	Particle size distribution/fiber length and diameter distributions
796.1550	Partition coefficient (n-octanol–water)
796.1570	Partition coefficient (n-octanol–water) — Estimation by liquid chromatography
796.1720	Octanol–water partition coefficient, generator column method
796.1840	Water solubility
796.1860	Water solubility (generator column method)
796.1950	Vapor pressure
Section No.	**Subpart C: Transport Processes Test**
796.2700	Soil thin layer chromatography
796.2750	Sediment and soil adsorption isotherm
Section No.	**Subpart D: Transformation Processes Test**
796.3100	Aerobic aquatic biodegradation
796.3140	Anaerobic biodegradability of organic chemicals
796.3180	Ready biodegradability: Modified AFNOR test
796.3200	Ready biodegradability: Closed bottle test
796.3220	Ready biodegradability: Modified MITI test (I)
796.3240	Ready biodegradability: Modified OECD screening test
796.3260	Ready biodegradability: Modified Sturm test
796.3300	Simulation test — Aerobic sewage treatment: Coupled units test
796.3340	Inherent biodegradability: Modified SCAS test
796.3360	Inherent biodegradability: Modified Zahn–Wellens test
796.3400	Inherent biodegradability in soil
796.3480	Complex formation ability in water
796.3500	Hydrolysis as a function of pH at 25 °C
796.3700	Photolysis in aqueous solution in sunlight
796.3780	Laboratory determination of the direct photolysis reaction quantum yield in aqueous solution and sunlight photolysis
796.3800	Gas phase absorption spectra and photolysis

[a] 40 CFR, Chapter 1, Part 796, July 1, 1990.

Water solubility, n-octanol/water partition coefficient, vapor pressure, dissociation constant, ultraviolet-visible (UV-vis) absorption spectrum, melting temperature, and density/relative density are some of the tests suggested for an understanding of the physical and chemical properties of a chemical and for defining the environmental fate and effects testing parameters.

TABLE 4

Environmental effects tests suggested by FDA for human animal health drugs and other FDA regulated chemicals[a]

Guideline No.	Test
4.01	Algal assay
4.02	Microbial growth inhibition
4.06	Seed germination and root elongation
4.07	Seeding growth
4.08	*Daphnia* acute toxicity
4.09	*Daphnia* chronic toxicity
4.10	*Hyalella azteca* acute toxicity
4.11	Freshwater fish acute toxicity
4.12	Earthworm subacute toxicity

[a] Environmental Assessment Technical Assistance Handbook, March, 1987.

TABLE 5

Environmental effects tests suggested by EPA under FIFRA[a]

Series No.	Test
Series 71: *Avian and Mammalian Testing*	
71-1	Avian single-dose oral LD50 test
71-2	Avian dietary LC50 test
71-3	Wild mammal toxicity test
71-4	Avian reproduction test
71-5	Simulated and actual field testing for mammals and birds
Series 72: *Aquatic Organism Testing*	
72-1	Acute toxicity test for freshwater fish
72-2	Acute toxicity test for freshwater aquatic invertebrates
72-3	Acute toxicity test for estuarine organisms
72-4	Fish early life-stage and aquatic invertebrate life-cycle studies
72-5	Life-cycle tests of fish
72-6	Aquatic organism bioavailability and accumulation tests
72-7	Field testing for aquatic organisms
Series 73: *Reptile and Amphibian Testing*	
73-1	Reptile single-dose oral LD50 test
73-2	Acute toxicity test with amphibians
Series 74: *Insect Pollinator Testing*	
74-1	Honey bee acute contact LD50
74-2	Honey bee — toxicity of residues on foliage
74-3	Field testing for pollinators

[a] Subdivision E, Wildlife and Aquatic Organisms, 1985.

TABLE 6

Environmental effects tests suggested by EPA Under the Toxic Substances Control Act[a]

Section No.	Subpart B: Aquatic Guidelines Test
797.1050	Algal acute toxicity test
797.1060	Freshwater algae acute toxicity test
797.1075	Freshwater and marine algae acute toxicity test
797.1160	Lemna acute toxicity test
797.1300	Daphnid acute toxicity test
797.1330	Daphnid chronic toxicity test
797.1350	Daphnid chronic toxicity test
797.1400	Fish acute toxicity test
797.1440	Fish acute toxicity test
797.1520	Fish bioconcentration test
797.1560	Fish bioconcentration test
797.1600	Fish early life stage toxicity test
797.1800	Oyster acute toxicity test
797.1830	Oyster bioconcentration test
797.1930	Mysid shrimp acute toxicity test
797.1950	Mysid shrimp chronic toxicity test
797.1970	Panaeid shrimp acute toxicity test
Section No.	**Subpart C: Terrestrial Guidelines Test**
797.2050	Avian dietary toxicity test
797.2130	Bobwhite reproduction test
797.2150	Mallard reproduction test
797.2175	Avian acute oral toxicity test
797.2750	Seed germination/root elongation toxicity test
797.2800	Early seedling growth toxicity test
797.2850	Plant uptake and translocation test

[a] 40 CFR, Chapter 1, Part 796, July 1, 1990.

Water solubility is an important basic property governing the tendency of a chemical to move and distribute between various environmental compartments and enter into living systems. Soluble chemicals are more likely to be distributed by the hydrologic cycle than relatively insoluble ones. In addition, water soluble chemicals are more likely to undergo, chemical, photochemical or microbial degradation because solubility facilitates better interaction with light (photodegradation), other chemicals (hydrolysis), and microorganisms. Water soluble chemicals are also less likely to become adsorbed to soils and sediments and less likely candidates for volatilization. Information on water solubility is important for proper design of other physical and chemical testing, as well as design and interpretation of fate and effects testing.

The n-octanol/water partition coefficient (K_{ow}) is important in assessing the fate of new chemicals. Based on the K_{ow}, the potential for the chemical to be

adsorbed to soil or sediment, and to become bioaccumulated in aquatic and terrestrial organisms can be estimated. Generally, a K_{ow} of less than 10 suggests low potential for accumulation in tissue, while a K_{ow} of greater than 10^6 suggests that the chemical will become accumulated in tissue; such accumulation could be potentially toxic to the test organisms and to the consumers of such organisms.

Vapor pressure governs the evaporative loss (volatilization) of a chemical. Volatilization is an important property governing the tendency of a chemical to be transported in air and is thus an important parameter in predicting the distribution of chemicals into environmental compartments. Highly volatile chemicals have a tendency to enter environmental compartments away from the site of intended or accidental release. The data on vapor pressure and water solubility permit the calculation of Henry's law constant, a parameter that is required for the calculation of volatility from water. In addition, chemicals with low vapor pressure, strong adsorption to soil or sediment, and high solubility in water are less likely to be airborne. Non-volatile chemicals are less likely to be transported and their fate in soils and water becomes a significant part of an EA. Information on vapor pressure should be obtained prior to design and conduct of other tests in order to prevent or account for loss of chemicals during the course of the test.

The *dissociation constant* is a measure of the degree of ionization of a chemical, which affects its availability to enter into physical, chemical, and biological reactions such as solubility, binding and membrane passage.

If a chemical absorbs radiation in the *ultraviolet-visible spectrum* (290–800 nm), then it may be susceptible to photodegradation. Photodegradation may occur in the aqueous as well as air and soil environments and UV-vis spectra appropriate to these media are needed to assess photodegradation and persistence of the chemical in these media.

The *melting temperature* of a chemical will indicate the physical state of a chemical at ambient temperature and thus some indication of the movement of a chemical within and between water, soil, and air.

The measurement of *density and relative density* (*specific gravity*) gives an indication of the movement of a chemical in the environment, i.e. sink, float or disperse in water.

2.3 Environmental fate testing

Chemicals undergo biological, chemical and photochemical transformations/degradations in the environment and data on such degradations are essential in estimating the expected environmental concentrations (EECs). Thus, EEC equals MEEC minus depletion due to transformation and degradation processes. Hydrolysis may be an important mode of chemical degradation for chemicals with hydrolysable functional groups such as esters, amides, alkyl halides, epoxides and phosphoric esters. Photodegradation may be important for chemicals that are directly released into the environment as well as pharmaceutical drugs that are exposed to light, e.g. in the aeration tanks in

WWTP. Chemicals that absorb light in the UV-vis spectrum may degrade photolytically in air, in water, or on soil.

Biological degradation is mediated by the microorganisms present in the water, air or soil. Microorganisms may have the ability to degrade the organic chemicals to inorganic components such as water and carbon dioxide. Often, the degradation may be partial resulting in metabolites that may be less readily biodegradable than the parent and may be less or more toxic. Such biodegradation may occur aerobically and/or anaerobically in water, soil, or sediment. The type of biodegradation testing should focus on the anticipated releases into specific environmental compartments and concentrations expected in these environmental compartments. For example, degradation in soil may assume special significance for agricultural chemicals with high potential for release into field soils, while degradation in wastewater may be important for pharmaceutical chemicals that are released into WWTP through human excreta. The degradation in marine sediment or water may be important for algicides (chemicals used to kill marine algae), or drugs used to control fish diseases.

In addition to the parent chemical, the chemical transformation or biological degradation products produced in the environment may need to be identified and tested for their potential toxic properties. The identification of the degradation products is more clearly mandated by FIFRA testing procedures than by TSCA or FDA. However, since identification of degradates and toxicity testing of such degradates to aquatic species are not excluded totally by TSCA regulations or FDA's testing requirements, it is important to consult the concerned regulatory agency for decision making in this area.

The mobility of a chemical is an important characteristic in establishing its potential for movement between environmental compartments through leaching, dissipation, volatilization and run-off. Adsorption/desorption, water solubility, and volatilization of the chemical will influence mobility, i.e. poor adsorption (high desorption), high water solubility, and volatility of a chemical will accentuate mobility.

There is some degree of commonality in the environmental fate testing guidelines of FDA (Table 1), and FIFRA (Table 2) and TSCA (Table 3). Each test is briefly described below.

Hydrolysis represents one of the most common and thus, one of the most potentially important pathways of degradation for many chemicals, as water is ubiquitous in all environmental compartments (air, terrestrial and aquatic). Hydrolysis data are important in the design and interpretation of fate and effects tests. For compounds undergoing hydrolysis, the transformation products may be of greater environmental concern than the parent compound, because the hydrolytic products may adversely affect non-target organisms.

Photodegradation represents another possible degradation pathway in water, soil, and air. Photodegradation may alter a chemical structure that is resistant to biodegradation or hydrolysis and thus provides a pathway for initial breakdown of some otherwise very stable chemicals. Photolysis rates

and half-lives can be determined and the persistence of the chemical and nature of the photoproducts formed are evaluated.

Adsorption/desorption processes affect the mobility and distribution of chemicals in the environment. If a chemical is strongly adsorbed to soil, it may be immobile and, therefore, will not be leached into ground water. If a chemical does not become adsorbed, it may spread to surface and ground water, accumulate in the water column, and affect aquatic organisms. The sorption and desorption of chemicals will also influence their potential to become photodegraded. In general, the rate of movement of a chemical is inversely correlated with adsorption. In addition to this study, leaching studies are listed under FIFRA guidelines in order to evaluate the potential leaching of pesticides and their degradates through the soil profile in terrestrial environments and to evaluate transport and dissipation in aquatic environments.

Knowledge of the *biodegradation* of a chemical is often critical in an assessment of its environmental exposure and impact because this may be the principal process by which it is reduced in complexity. The test for biodegradation in water is important for pharmaceutical chemicals due to the potential of their degradation in WWTP. Biodegradation in soil is an important degradation pathway for industrial and agricultural chemicals and animal health drugs. The mobility of the chemicals from soil into aquatic environments may necessitate the study of biodegradation in sediment. Biodegradation rates can be measured using methods outlined in the TSCA and FDA environmental guidelines.

FIFRA guidelines suggest that studies of *aerobic and anaerobic soil metabolism* should be conducted to access the nature and extent of degradation products. In agricultural cropping practice, the rotational crops that follow a target crop to which the pesticide is applied are potentially exposed to chemicals and their metabolites remaining in soils. Non-target organisms may also be exposed. Aquatic environments may be contaminated with pesticides applied to agricultural crops through runoff from agricultural fields or through direct aquatic use.

Field volatility studies are suggested for chemicals which have very high volatility potential, because vapors resulting from pesticides applied to agricultural crops can cause inhalation exposure to man as well as to non-target organisms.

Field dissipation studies are suggested by FIFRA guidelines for pesticides used in terrestrial and aquatic environments. The objective of these studies is to estimate the extent of residue dissipation under actual field use. The mobility, degradation and dissipation of pesticides are evaluated in the field dissipation studies. Dissipation studies may also be required for tank mixes or combinations of pesticides to assess overall persistence in the soil. Long term soil dissipation studies are required for those pesticides that dissipate slowly.

Confined and field accumulation studies are suggested for determining the amount and nature of pesticide residues taken up by rotational crops. Confined accumulation studies are done in the laboratory, greenhouse, or outdoor small

plots to obtain preliminary information on residue uptake. The field accumulation studies are conducted to determine the residue uptake by rotational crops under actual field-use conditions. Special studies on accumulation in irrigated crops may be required to assess residue uptake by upland crops, e.g. when reclaimed waste water or water from rice fields is used to irrigate upland crops.

2.4 Environmental effects testing

A significant component of the testing for an EA is the determination of a chemical's effect on the terrestrial and aquatic communities present in the receiving environmental compartments. Both acute and chronic tests may be required. Chemicals that have a high potential to become biodegraded and are, therefore, short-lived in the environment may require only acute toxicity testing for non-human biota in the ecosystem. Chemicals that tend to persist (non-biodegradable and/or tightly bound to soil or sediment) and thus accumulate with time may require chronic toxicity testing for developmental and reproductive effects. Long term toxicity, bioaccumulation and bioconcentration tests may also be required. In addition, toxicity to common microorganisms present in the terrestrial and aquatic environments is important because of the important role microorganisms play in the biogeochemical cycles of the ecosystem (carbon, nitrogen, sulfur cycles etc.). The EECs should be taken into consideration in the interpretation and assessment of environmental significance of the toxicity observed in any of the effects tests.

Results from effects testing are used to help predict the effects, if any, that a compound may have on the biological communities and specific organisms in the receiving environment. Selection of tests should take into account how the compound will be used and which environments will receive it. The effects testing suggested for FDA, FIFRA and TSCA is presented in Tables 4, 5, and 6, respectively. A summary of major tests is provided below.

The possible effects of a compound, both inhibitory and stimulatory, on the growth rate and biomass of algae (*algal toxicity*) is important because these organisms serve as a foundation of most aquatic food chains. Algae also carry out a significant percentage of all photosynthesis on earth and aid in transforming nitrogenous wastes to innocuous effluents.

The *microbial growth inhibition test* determines the sensitivity of cultures of bacteria, fungi, and bluegreen algae to a compound. Significant microbial growth inhibition may result in reduction of plant growth or quality and interference with the natural degradative functions of microorganisms.

Possible effects of a compound on plant life can be assessed by tests for seed germination, root elongation, and seedling growth (*non-target plant tests*). Inhibitory or stimulatory effects can alter plant's reproductive success, competitive relationships, or overall productivity.

The *Daphnia acute and chronic toxicity tests* are designed to assess the effects of a test compound on the survival of *Daphnia* as a representative planktonic macroinvertebrate. Acute tests are valuable for supplying a rapid estimate of

the relative toxicity of a test compound and are usually simpler and less costly to perform than a chronic test.

Acute toxicity to Hyallella azteca, a freshwater amphipod crustacean, may be particularly useful for determining the possible toxicity of compounds that partition to sediments or adsorb onto the surfaces of aquatic plants.

Fish are important and desirable test organisms since they are widely distributed throughout most aquatic environments and are an essential link in converting aquatic matter into human or animal food (*Fish Toxicity Test*). Rainbow trout (*Salmo gairdneri*) and bluegill (*Lepomis macrochirus*) are the two freshwater species most frequently used in acute toxicity tests as representative cold and warm water species, respectively.

The earthworm is the preferred test organism for determining toxicity to a terrestrial species. They are ubiquitous throughout many soil types, ecologically important, and an integral part of the food chain (*Earthworm Toxicity Test*). Because the test compound is mixed with soil in the test, an indication of toxicity associated with passage through the gut of a higher organism can be gained.

The environmental effects testing suggested by FIFRA covers avian, mammalian, and aquatic organisms, reptiles and amphibians, and insects. The environmental effects testing suggested by TSCA is organized under aquatic and terrestrial guidelines. In addition to fish testing, acute and chronic toxicity testing plus bioconcentration determinations with shrimp may be required depending on the use patterns of the chemical and potential entry into aquatic compartments. The terrestrial guidelines include seed germination/root elongation, early seedling growth toxicity, and plant uptake and translocation tests.

3. Tiered testing approach

To assist industry in developing appropriate and efficient testing programs, a four-tier Environmental Assessment Technical Test Matrix (Matrix) has been devised by the Pharmaceutical Manufacturer's Association [2]. This Matrix supplements the TAH and is primarily intended to provide clarification to the pharmaceutical industry on EA requirements for human drugs, but the approach is relevant to other industries and chemical types. The following information is taken from this document which is entitled "Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA".

The Matrix is not intended to represent the chronology of testing but rather the logic. It is composed of four Tiers of which Tier 0 presents the minimum base set of tests needed to determine the environmental compartments most likely to be affected by the target compound and the criterion values for determining which additional Tier or Tiers to include. Test methods should be those provided in the TAH, or the equivalent, and fulfill FDA reporting requirements.

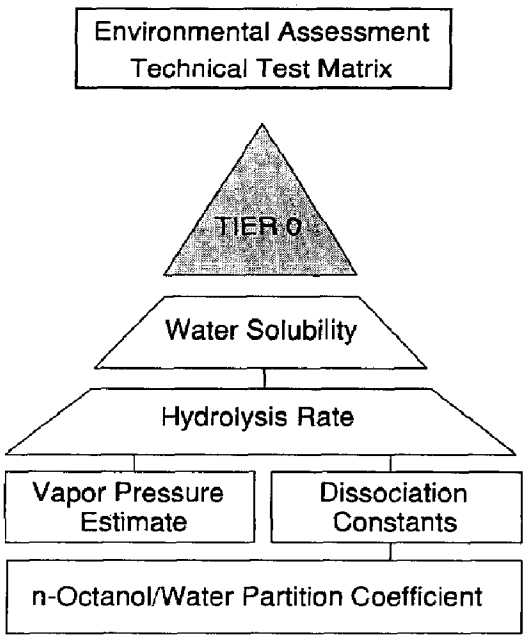

Fig. 1. Environmental Assessment Technical Test Matrix — Tier 0.

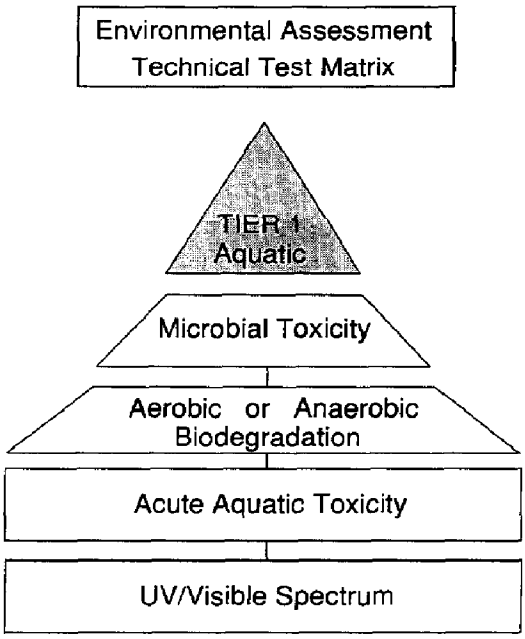

Fig. 2. Environmental Assessment Technical Test Matrix — Tier 1.

Tier 0 (Fig. 1) provides guidance as to which subsequent Tiers are most likely to be appropriate.

(1) Determine the water solubility and hydrolytic stability of the test compound. If the compound is hydrolysed at such a rate that after five days, the concentration is less than 90% of the initial concentration, then hydrolysis may be considered a primary removal process, and additional studies will depend on the nature of the hydrolysis products.
(2) Determine the dissociation constant(s) of the compound.
(3) Determine the octanol/water partition coefficient of the compound. The base 10 logarithm of this value, called log K_{ow} (or log P) is a commonly used parameter for predicting environmental fate.
(4) Estimate the vapor pressure of the compound. If the estimate is greater than 10^{-7} Torr, then the actual vapor pressure must be determined experimentally.
(5) Based on the data obtained from this preliminary set of tests, decide which additional tests in which Tiers are needed.

Tier 1 (Fig. 2) provides guidance logic for the aquatic environmental compartment. This is the compartment in which most human drug substances will be found, due to passage through a waste water treatment plant.

(1) A key test for human drugs is the determination of the biodegradability in water of the test chemical since the result relates to emission from a waste water treatment plant and persistence in the environment.

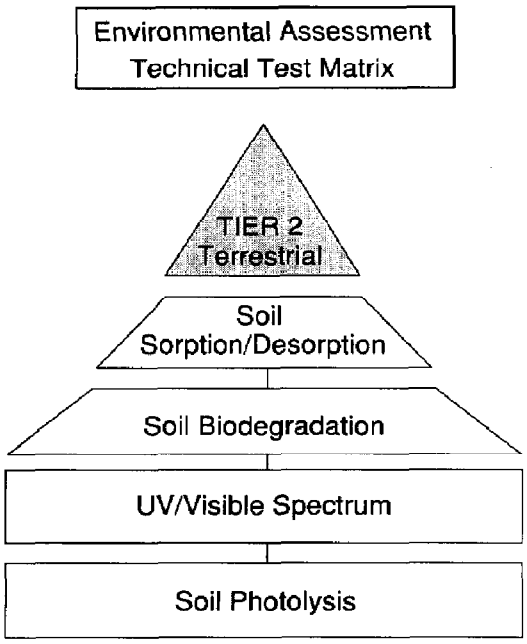

Fig. 3. Environmental Assessment Technical Test Matrix — Tier 2.

(2) If the compound is not readily biodegraded, it may be useful to calculate the Expected Environmental Concentration (EEC) of the compound, subtract depletion due to biodegradation processes, and compare this value with a toxicity measure. For human drugs, calculation of the maximum EEC can be found in the PMA Guidance Document [2]. For animal drugs, construction of a worst-case scenario will be helpful.

A key toxicity test for the aquatic compartment is Acute Aquatic Toxicity, e.g. to *Daphnia*. If the EEC is shown to be less than 1% of the acute toxicity measured for the compound, then additional testing may not be required. If the EEC criterion cannot be met, then investigation into other possible depletion processes should be considered.

(3) Determination of the potential photolytic decomposition may be determined from the UV–Vis spectrum of the compound. If there is significant absorption at wavelengths greater than 290 nm, then determination of the rate of aqueous photolysis should be considered. If the half-life is less than five days, additional testing may not be required.

Tier 2 (Fig. 3) presents the logic matrix for the terrestrial environmental compartment.

For compounds which are not readily biodegraded, the sorption coefficient (K_{oc}) is used as a criterion for predicting whether or not a human drug will sorb to sewage sludge or remain in the aqueous effluent from the waste water treatment plant. If the K_{oc} is approximately or greater than 100, then fate in the terrestrial environmental compartment should be considered and soil biodegradation may become an important depletion mechanism. As for Tier 1, other

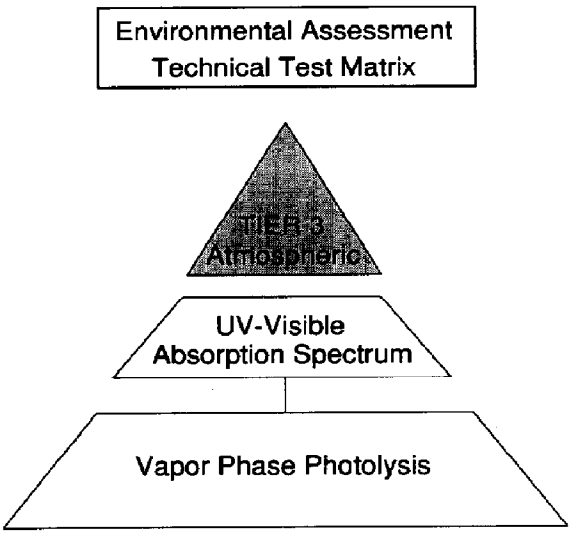

Fig. 4. Environmental Assessment Technical Test Matrix — Tier 3.

tests may be required to ascertain expected environmental fate, and calculation of the EEC and comparison with a toxicity measure may be useful.

Tier 3 (Fig. 4) presents the logic matrix for the atmospheric environmental compartment.

In this compartment, the requisite data include the UV–Vis spectrum, and if there is significant absorbance and volatility, a determination of the rate of vapor phase photolysis may be advisable. Again, it may be useful to calculate the EEC of the compound and compare it with an appropriate toxicity measure.

In summary, if the following basic criteria are met, further testing may not be required: (1) log K_{ow} (or log P), the octanol–water partition coefficient, is less than 2; (2) vapor pressure is less than 10^{-7}; (3) water solubility is greater than 500 ppm; (4) biodegradation half-life is less than 8 hours or less than 28 days and K_{oc} (sorption coefficient) is less than 100; and (5) EEC is less than 1% of an appropriate toxicity measure.

4. Conclusions

While the basic objective of laboratory and field testing for support of an EA for FDA and EPA regulated chemicals is similar, the appropriate studies for an EA are driven by the potential use and exposure patterns of the test chemical. A testing strategy should be established and discussed with the concerned regulatory agency at an early point in the development cycle of the potential product. Proceeding according to the logic of the above described Matrix should provide the most efficient and economical testing strategy while still providing sufficient relevant information to regulatory agencies on the

environmental fate and effects of a chemical. Depending on results from the test Matrix, additional tests may be required.

It is recommended that the appropriate regulatory agency be regularly consulted throughout the testing process for guidance and that protocols for non-standardized environmental tests be submitted for regulatory review before test initiation. If an outside organization is contracted for studies, it is recommended that it be certified for experience with appropriate test procedures, GLP compliance, and analytical capabilities.

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