Slide	Narration- Environmental Fate and Transport
1	Hello and welcome to the training series on Data Requirements for Antimicrobial Pesticides, 40 CFR part 158 subpart W, also referred to as 158W. This presentation addresses key concepts about environmental fate and transport processes and associated data requirements.
2	Before 158W, the Office of Chemical Safety and Pollution Prevention, which includes the Office of Pesticides Programs, characterized many antimicrobial uses as “indoor uses” and assumed that potential for release to environmental media from these uses would be negligible. Consequently, although many of these uses had the potential to be released “down-the-drain” to wastewater treatment plants and subsequently be discharged to surface water, potential risks to aquatic organisms and humans from exposure to surface water downstream of wastewater treatment plants were not being considered. With the promulgation of 158W, the Agency will assess potential environmental exposures and risks to aquatic organisms and humans resulting from exposure to antimicrobial pesticides released “down-the-drain.” As a result, some new data concerning environmental fate and effects during wastewater treatment will be required to enable the Agency to estimate these potential exposures and risks from “down-the-drain” releases. Down-the-drain releases are those releases of antimicrobial pesticides that originate from residential, commercial, institutional, and industrial sources, are discharged to wastewater treatment plants, and may subsequently enter surface water downstream of wastewater treatment facilities. Examples of products containing antimicrobials that have the potential to be released down-the-drain include toilet bowl cleaners and laundry detergents. One purpose of this presentation is to provide a broad overview of environmental fate and transport processes, including chemical degradation, microbial degradation, intermedia transport, and fate in wastewater treatment plants, also referred to as WWTPs. Another purpose is to provide information on the role of physical/chemical properties and WWTP effects data in determining environmental fate data requirements for antimicrobial pesticides.
3	Environmental fate data provide information on a chemical’s persistence in soils, aquatic sediments, and surface water under aerobic and anaerobic conditions. These data also provide information on sorption, or attachment, to soil and sediment. In addition, environmental fate data provide information on the tendency of an antimicrobial pesticide to leach from treated wood, antifoulant paints applied to boats, and to be released with ballast water discharges to the aquatic environment. Wastewater treatment plant data on fate and effects include information on biodegradation during wastewater treatment, sorption to activated sludge, and toxicity to activated sludge microorganisms.
4	The elements of an environmental fate assessment are: chemical or abiotic degradation, microbial or biotic degradation, and intermedia transport. Chemical degradation occurs in environmental media such as air, water, soil, and sediment. Microbial degradation is

	assessed in water, soil and sediment. Intermedia transport is evaluated in environmental media and can occur by processes including leaching from surfaces such as wood or antifoulant paint, during wastewater treatment, and in release from waste water treatment plants. Antifoulant paints, wood preservatives and any product that may go down the drain may require more environmental fate data than other end-uses.
5	This slide presents the 12 major use patterns for antimicrobial pesticides. These include: - Agricultural Premises and Equipment; - Food Handling and Storage Establishments, Premises and Equipment; - Commercial, Institutional and Industrial Premises and Equipment; - Residential and Public Access Premises; - Medical Premises and Equipment; - Human Drinking Water Systems; - Materials Preservatives; - Industrial Processes and Water Systems; - Antifoulant Coatings and Ballast Water Treatments; - Wood Preservatives; - Swimming Pools and Spas; - Aquatic Areas
6	The 12 antimicrobial use patterns were further grouped into 5 major use sites: Industrial processes and water systems (which is use pattern number 8), Antifoulant coatings and paints (which is major use pattern number 9 and also includes ballast water treatments), Wood preservatives (which is major use pattern number 10), aquatic areas (which is major use pattern number 12 and involves direct application of antimicrobial products to the aquatic environment, and the catch-all category, all other use patterns (which includes use patterns 1 through 7 and 11).
7	There are 2 major types of degradation processes: abiotic or purely chemical processes and biotic processes which are the result of microbial metabolism. There are three chemical degradation studies: Hydrolysis, which is addressed in OCSPP guideline number 835.2120; Photodegradation in water, which is addressed in OCSPP guideline number 835.2240; and Photo-degradation on soil, which is addressed in OCSPP guideline number 835.2410.
8	The study of hydrolysis in the absence of light is required for all pesticides and measures abiotic degradation. In OCSPP guideline studies, hydrolysis is conducted in water at pH 5, 7, and 9. In OECD guideline studies, hydrolysis is conducted in water at pH 4, 7, and 9. These pH ranges are intended to represent pH values typical of the ambient environment. These studies not only measure persistence and the half-life of an antimicrobial in water, but also identify degradation products, their half-lives, and information on rates of formation and decline rates of the antimicrobial and its degradation products.
9	Photodegradation in water measures degradation by sunlight at a pH at which the compound is hydrolytically stable. Specifically, it measures photodegradation half-lives and identifies photodegradation products and their formation and decline rates.

	This study may be waived if the compound does not absorb ultraviolet light between 290 and 800 nanometers (nm) in wavelength. This 290-800 nm range represents the wavelength range for sunlight. The study may also be waived if the antimicrobial is not stable at pH 5, 7, or 9 in the hydrolysis study.
10	The photodegradation in soil guideline study measures degradation of a chemical on soil surfaces by sunlight. It identifies photodegradation half-lives, photodegradation products formed, and their formation and decline rates. This study may be waived if the antimicrobial does not absorb ultraviolet, or visible light, between 290 and 800 nm in wavelength.
11	This slide presents the relative differences in the elements in which abiotic studies are conducted. The chemical or abiotic studies are listed in order of complexity with regard to elements in which they are tested. Note that the hydrolysis study is conducted using sterile buffered solution in the absence of light. Photodegradation in water studies should be conducted at a pH at which the compound is the most hydrolytically stable. The sterile buffered samples are exposed to a light source that represents sunlight. Photodegradation on soil studies are used to determine if a compound will photodegrade on solid surfaces such as soil and are conducted using water, light, and soil.
12	This slide discusses microbial degradation. There are 4 different microbial degradation studies including aerobic soil metabolism, anaerobic soil metabolism, aerobic aquatic metabolism and anaerobic aquatic metabolism. Aerobic studies are conducted in the presence of oxygen. Anaerobic tests are conducted in the absence of oxygen by introducing nitrogen gas in place of oxygen. The aerobic aquatic metabolism study has the shortest test duration, from 30 to 60 days. The other biotic tests are conducted for a period of up to one year. The soil metabolism studies measure potential degradation and sorption in the soil environment. The aquatic metabolism studies measure degradation and sorption in water:sediment systems. Note that all of these laboratory studies are conducted in the absence of light. In aquatic metabolism studies, flooded soil is a key component. Flooded soil refers to soil that is saturated with water and has a layer of water over it. It represents the bottom sediment in a water:sediment system. Nitrogen is used in anaerobic metabolism tests to simulate the relative lack of oxygen in the benthic or bottom sediment compared to the water column, that is the stream, lake, or other water body. Oxygen is used in aerobic metabolism studies to create an aerobic environment.
13	This slide discusses the aerobic soil metabolism study. This study approximates conditions on the top layer of soil after rain and subsequent drying-out. It measures degradation of parent compound in soil in the presence of oxygen; that is, in the soil near the surface. It also measures formation and decline rates of degradation products.

14	This slide discusses the anaerobic soil metabolism study. This study represents the metabolism of a pesticide in soil in the absence of oxygen, as might occur in the top layer of soil following a rainfall event or in lower or saturated soil strata, which is simulated by saturating the soil with an inert gas such as nitrogen. It measures degradation of the parent compound and formation and decline rates of degradation products.
15	This slide discusses the aerobic aquatic metabolism study. This study represents metabolism in surface water and is conducted in water with some sediment in the presence of oxygen. It measures degradation of the parent compound and formation and decline rates of degradation products. Additionally, it measures partitioning of the parent compound and degradation products between water and sediment.
16	This slides discusses the anaerobic aquatic metabolism study. This study represents metabolism occurring in the suspended and bottom sediment in a body of water. It measures degradation of the parent compound and formation and decline rates of degradation products. It also measures partitioning of the parent compound and degradation products between water and sediment.
17	This slide summarizes key features of the four biotic degradation studies. It is important to note that these four microbial degradation studies are all laboratory studies conducted in darkness. The relative complexity of the test medium is presented in this table. Aerobic soil metabolism studies involve the presence of water, soil, and oxygen but no flooding of soil and no saturation with nitrogen. An anaerobic soil metabolism study initially involves soil, water and oxygen, and nitrogen is added after a half-life of the test substance has been established. Anaerobic aquatic metabolism studies involve soil, water, flooding, and nitrogen, but no oxygen. Aerobic aquatic metabolism studies involve soil, water, flooding, and oxygen, but no nitrogen.
18	There are two more important fate studies: these are soil leaching and adsorption/desorption studies. Soil leaching studies are conducted using the parent compound and measure the distance a pesticide can leach or move into or through the soil. The adsorption/desorption study assesses the affinity of the parent compound for soil particles, which is reported as the Kd or Koc value. The higher the value, the stronger the predicted binding capacity and the lower the potential for migration.
19	Studies for which no OCSPP guidelines are available are characterized as non-guideline studies. Three of these studies are intended to measure leaching from a substrate to which a product containing an antimicrobial pesticide is applied or incorporated. One non-guideline leaching study is used to measure leaching of antifoulants used as materials preservatives in paints applied to boat bottoms. In the absence of an OCSPP guideline study, there is a study developed by the American Society for Testing Materials, or ASTM, that measures the rate of antimicrobial pesticide release to water from the paint applied to a ship or boat bottom.

	There are two non-guideline studies that measure leaching of antimicrobial pesticides such as wood preservatives. These studies were developed by the American Wood Preservers Association, also known as AWPA. AWPA E20-08 measures leaching from treated wood that may come into contact with soil, such as utility poles. AWPA E 11-12 measures leaching from treated wood that may come into contact with water, such as pilings for piers. Exposure to antimicrobial pesticides that may leach from textiles and plastics may be a potential concern. Although a study to measure the leaching of antimicrobials such as materials preservatives in textiles and plastics is not required by the Agency under 158W, in the absence of these data, EPA makes a conservative assumption that 100% of the antimicrobial pesticide leaches from the textile or plastic material. Since there is no set protocol for these studies, EPA encourages registrants to submit protocols to the Agency for review in the hope that a methodology can be developed that will generate acceptable data that may allow the Agency to assess potential exposures from leaching of antimicrobials from materials preservatives less conservatively.
20	Another important end-use of antimicrobials is as a biocide in the treatment of ballast water. Ballast water is “fresh or salt water, sometimes containing sediments, that is held in tanks and cargo holds of ships to increase stability and maneuverability during transit”. Water taken from one body of water and discharged into another water body can introduce invasive or non-native species of aquatic life from one water body to the other. The ballast water must be treated with antimicrobials prior to release from ships to prevent the spread of invasive or non-native species. Although there are no studies that are specific for ballast water assessment, any of the various abiotic and biotic laboratory studies that are able to demonstrate the degradation of the active ingredient and measure the formation and decline of the degradates may inform the ballast water assessment. EPA will model the estimated environmental concentration, or EEC, of pesticides in lakes or reservoirs taking into account the amount of antimicrobial released to the water body and the size of the receiving water body. From these EEC values, risk quotients are calculated to predict potential risk to the aquatic species in the vicinity of the ballast water discharge.
21	Another important area of consideration for antimicrobial pesticides is the potential environmental fate and effects of antimicrobials in wastewater treatment plants, referred to as WWTPs. Why are WWTP fate and effects data needed? One reason is to assess the potential effect of the antimicrobial on the microorganisms in the most sensitive biological treatment processes of a WWTP. Another is to assess the potential for the antimicrobial chemical to pass through the WWTP in the effluent to surface water where aquatic organisms and humans may be exposed; this type of analysis is commonly referred to as a down-the-drain assessment. The goal of data required for down-the-drain assessment is to determine toxicity to activated sludge microorganisms and percent removal of a chemical substance during wastewater treatment.

	Activated sludge is a biological wastewater treatment process that uses microorganisms to feed on organic contaminants in wastewater to reduce the levels of chemical substances in wastewater treatment plant effluent. The activated sludge process is not the only type of biological treatment process, but it is the most common type employed in the United States. It is widely used by large cities and communities where large volumes of wastewater must be treated economically. Private residences, commercial establishments such as hotels and restaurants as well as institutional establishments such as hospitals may also discharge down-the-drain to wastewater treatment plants that employ the activated sludge treatment process. WWTP data are needed to provide more refined, less conservative, exposure estimates. In summary, these data requirements are not meant to be a burden. It is to the benefit of registrants to provide these data to enable the agency to estimate exposure less conservatively.
22	Three notable definitions of biodegradation include a simple definition, one by EPA, and one by USGS. The simple definition of biodegradation is "the destruction of organic compounds by microorganisms". One way EPA has defined biodegradation is as, "A process by which microorganisms transform or alter, through metabolic or enzymatic action, the structure of chemicals introduced into the environment". USGS has defined biodegradation as "Transformation of a substance into new compounds through biochemical reactions or the actions of microorganisms such as bacteria."
23	This slide summarizes the concept of the environmental transport process, sorption. In a wastewater treatment plant, sorption leads to a transfer of chemical substance from the aqueous phase of wastewater treatment plant influent to sludge. Sludge, also referred to as biosolids, refers to the solids that settle out during the activated sludge treatment process. An activated sludge sorption isotherm study measures the extent to which a chemical substance distributes itself between activated sludge as the sorbent and water as the solvent. The equation describing this relationship is called a sorption isotherm. If a chemical substance is sorbed to sludge biomass, it may be removed from WWTP systems along with other solids by clarification. If a chemical substance is not sorbed, it will remain in the aqueous phase where it is subject to degradation or removal via biodegradation, chemical interactions, and/or volatilization. Sometimes a chemical is not removed by any of these fate and transport processes.
24	This slide lists 11 new data requirements under 158 W. Six of these are wastewater treatment plant data requirements. One of these tests, activated sludge respiration inhibition, measures effects to WWTP microorganisms. Tests 4 through 8 on this list pertain to fate and transport in a waste water treatment plant.

25	There are 3 basic types of wastewater treatment plant data requirements. One is fulfilled by a test to measure effects of antimicrobials on WWTP microorganisms. This test, an activated sludge respiration inhibition study, assesses toxicity of chemical substances to activated sludge microorganisms. The second type of data requirement determines the potential of a pesticide and/or its degradates to biodegrade during wastewater treatment. Biodegradation during wastewater treatment can be determined from a ready biodegradability test or a biodegradation in activated sludge simulation test. A third type of test, an activated sludge sorption isotherm study, measures the potential for a chemical substance to sorb to activated sludge. As mentioned earlier, biodegradation and sorption are key processes by which antimicrobials can be removed from wastewater. In addition to information on removal of an antimicrobial during wastewater treatment, it is also important to consider the possibility that the parent antimicrobial may biodegrade and/or hydrolyze rapidly. For instance, hydrolysis or biodegradation of an antimicrobial may be rapid enough that the antimicrobial may not persist long enough for aquatic organisms downstream of a wastewater treatment plant to be exposed to the parent chemical. At the same time, It is also important to determine whether degradation of the parent antimicrobial may form degradates that may be persistent and potentially toxic to aquatic organisms.
26	This slide summarizes new WWTP data requirements. These generally include three studies. One study, the Activated Sludge Respiration Inhibition or ASRI study, measures toxicity to activated sludge microorganisms. A second study is used to determine biodegradability during wastewater treatment. Here the Registrant can choose one of four studies depending on toxicity to microorganisms, as determined from the results of the ASRI study. A third study that is required, the Activated Sludge Sorption Isotherm or ASSI study, is used to determine what sorbs or adheres to activated sludge and what stays in the liquid phase.
27	This slide presents the decision tree that describes the WWTP fate and effects testing scheme. The key at the upper left hand corner of this slide includes several abbreviations needed to follow this decision tree. The abbreviation ASRI stands for Activated Sludge Respiration Inhibition study. ASSI stands for Activated Sludge Sorption Isotherm study. BAS stands for Biodegradation in Activated Sludge, which can be one of two biodegradation simulation tests. Note that the Porous Pot study found in this decision tree is also a biodegradation simulation study. The term in this decision tree, Ready Bio, stands for Ready Biodegradability Study. One thing to note when looking though the decision tree is that EC50 is the concentration of a chemical that exhibits an inhibitory or toxic effect on half of the microorganisms in a population. The lower the EC50 value, the higher the toxicity.

	The first step for the decision tree is to perform an ASRI study. Depending on the EC50 result from this study, there are 2 different paths that can be taken: one for if the EC50 from the ASRI study is less than or equal to 20 milligrams per liter and another if the EC50 from the ASRI study is greater than 20 mg/L If the EC50 from the ASRI study is less than or equal to 20 milligrams per liter, then the agency would generally require an ASSI test and one of two BAS studies or a Porous Pot test. The Agency determines the percent removal during waste water treatment to use in the down-the-drain analysis based on the results from the ASSI study and the appropriate WWTP biodegradation study. If the EC50 from the ASRI study is greater than 20 milligrams per liter, there are two subsequent choices. One choice is to conduct one of three biodegradation simulation studies. These will be identified later in this presentation. The second choice is to conduct a ready biodegradability study. If one chooses to conduct a ready biodegradability study and the chemical passes that study, the Agency would use those results for percent of chemical removed by biodegradation during wastewater treatment and no other testing would be required. If, however, the chemical fails the ready biodegradability study, then EPA will require the activated sludge sorption isotherm study, or ASSI and one of the three biodegradation simulation tests. The results of these tests would be used to determine percent removal of a chemical during wastewater treatment via sorption and biodegradation.
28	In these next few slides, I'll be discussing in more detail why the Agency requires each of the wastewater treatment plant studies that are in 158W. Why is an Activated Sludge Respiration Inhibition or ASRI study required? This test is required to determine the toxicity of a chemical substance to activated sludge microorganisms; such toxicity can inhibit the ability of activated sludge microorganisms to remove organic matter and treat wastewater. Another reason the ASRI study is required is to determine whether a ready biodegradability study will be adequate or a biodegradation simulation test will be required. If a chemical substance is too toxic to microorganisms, the performance of the ready biodegradability study will be compromised. The protocol for the ready biodegradability study states that chemical substances with EC_{50} values of less than or equal to 20 milligrams per liter are likely to pose serious problems. This stated likelihood for chemicals with EC50 values of less than or equal to 20 mg/L to pose problems in the ready biodegradability study forms the basis for establishing the 20 mg/L threshold to determine the type of WWTP biodegradation study to require.
29	What Waste Water Treatment Plant biodegradation tests are required under 158W? Tests required are a ready biodegradability test or one of three simulation tests for biodegradation in activated sludge. These include: Simulation Tests to assess the biodegradability of chemicals discharged in wastewater, OCSPP 835.3280; Simulation Test -

	Aerobic Sewage Treatment: Activated Sludge Units, OCSPP 835.3240; or a Porous Pot Test, OCSPP 835.3220.
30	What are key elements of a ready biodegradability test versus a biodegradation simulation test? In a ready biodegradability test, a solution of test substance is inoculated with microorganisms and incubated under aerobic conditions in the dark or in diffuse light. Parameters such as dissolved organic carbon, carbon dioxide production, and oxygen uptake are measured at intervals that are frequent enough to allow identification of the beginning and end of biodegradation. Normally, the test lasts for 28 days. A chemical passes the test if 70% of Dissolved Organic Carbon is removed in a 10-day window within the 28-day period of the test. For a respirometric test, the chemical must achieve 60% of the theoretical oxygen or theoretical carbon dioxide production. There are 6 different ready biodegradability tests identified in the OCSPP test guideline 835.3110: -DOC die-away -CO2 evolution, which is respirometric -MITI, which is respirometric -Closed bottle, which is respirometric -Modified OECD screening -Manometric respirometry There is information in the guideline to help one determine which of these tests would be the best choice based on properties of the chemical being tested. In contrast, a biodegradation simulation test is designed to determine the elimination and primary and/or ultimate biodegradation of water-soluble organic substances by aerobic microorganisms in a continuously-operated test system that simulates the activated sludge process. The test method is designed to ascertain whether the chemical tested can be biodegraded within the limits imposed by typical WWTPs. Because this is a simulation test, it is assumed that results can be extrapolated to predict elimination in full-scale treatment plants.
31	How does one know whether a simulation test rather than a ready biodegradation test is required? As mentioned earlier in this presentation, if the result of the ASRI test is an EC_{50} less than or equal to 20 mg/L, a simulation test is required. If the result of the ASRI test is an EC_{50} greater than 20 mg/L, one can perform a ready biodegradability test or a biodegradation simulation test; if, however, in this latter case the chemical fails the ready biodegradability test, a biodegradation simulation test is required.
32	When is a WWTP biodegradation test not required? A WWTP biodegradation test would not be required when a chemical substance is classified as a metal. This is because metals do not degrade.

	A WWTP biodegradation test would not be required when a chemical is relatively volatile, but not hydrophobic. Hydrophobic compounds repel water or tend to fail to mix with water (e.g., oil). A WWTP biodegradation test would not be required when a chemical is highly reactive. Another instance when a WWTP biodegradation test would not be required is when both the parent chemical and all of its degradation products have half-lives of less than 3 hours. Such chemicals would be expected to virtually disappear while travelling from the source to the wastewater treatment plant intake. Even if such a chemical reaches a wastewater treatment plant, it would not be expected to be discharged to surface water since it would not persist long enough. A final example of an instance in which a WWTP biodegradation test would not be required is when none of the registered or proposed product uses would result in transport of the parent and/or its degradation products to a wastewater treatment plant.
33	Under what circumstances is an activated sludge sorption isotherm test, or ASSI test, not required? One instance would be If an antimicrobial is relatively volatile, but not hydrophobic. Another instance would be if an antimicrobial is highly reactive. Yet another case would be if the log $K_{ow,}$ which is a measure of the octanol /water partition coefficient, is less than 3.0. K_{ow} is the ratio of the chemical's concentration in n-octanol to its concentration in a known volume of water after the octanol and water have reached equilibrium. The higher the K_{ow} value, the higher the tendency to sorb to soil-type solids rather than to remain in water (the aqueous phase)
34	What criteria trigger the requirement for an ASSI test? If the criteria for not requiring this test are not met, then the ASSI test is required if one or more of the following criteria are met: The antimicrobial is a metal; there are exceptions such as copper and silver. Copper and silver have wastewater treatment plant removal data from EPA WWTP studies. The log K_{ow} is greater than or equal to 3.0. Another criterion is that the antimicrobial is polycationic. A polycation is a molecule or chemical complex having positive charges at several sites. Yet another criterion is that the EC_{50} in the activated sludge respiration inhibition test is less than or equal to 20 mg/L.

	Still another criterion is that the EC_{50} in the activated sludge respiration inhibition test is greater than 20 mg/L, but the antimicrobial fails the ready biodegradability test.
35	In summary, data from environmental fate studies are not intended to increase economic burden; they are intended to provide better and less conservative estimates of exposure. The chemical and microbial degradation tests, the transport and mobility studies, including leaching studies, and the WWTP studies are intended to inform a more realistic exposure assessment than could be conducted using default assumptions. Only data for the particular chemical properties and use patterns that are needed to assess risk are required. Note that the environmental fate data requirements table of section 158.2280, subpart W, contains test notes with detailed information on when specific fate studies are and are not required.