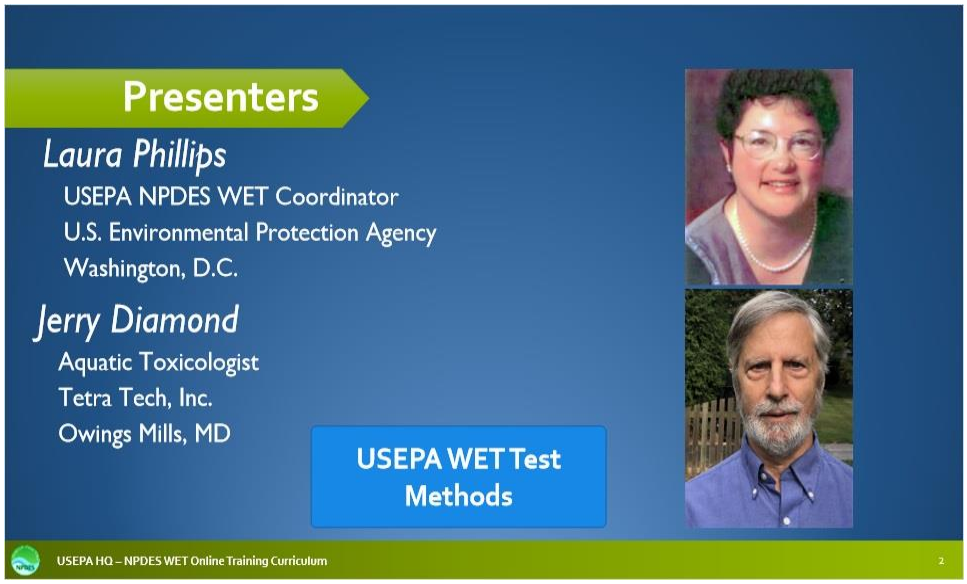
Module 10 – USEPA NPDES Toxicity Reduction Evaluations (TREs) and Toxicity Identification Evaluations (TIEs)

Notes:

Welcome to this presentation on the United States Environmental Protection Agency, hereafter USEPA, National Pollutant Discharge Elimination System, or NPDES, Toxicity Reduction Evaluations and Toxicity Identification Evaluations. This presentation is part of a web-based training series on Whole Effluent Toxicity, or WET, sponsored by the USEPA Office of Wastewater Management's Water Permits Division.

You can review this stand-alone presentation, or, if you have not already done so, you might also be interested in viewing the other presentations in the series, which cover the use of WET in NPDES permit program.

Before we get started with this presentation, I'll make some introductions and cover two important housekeeping items.



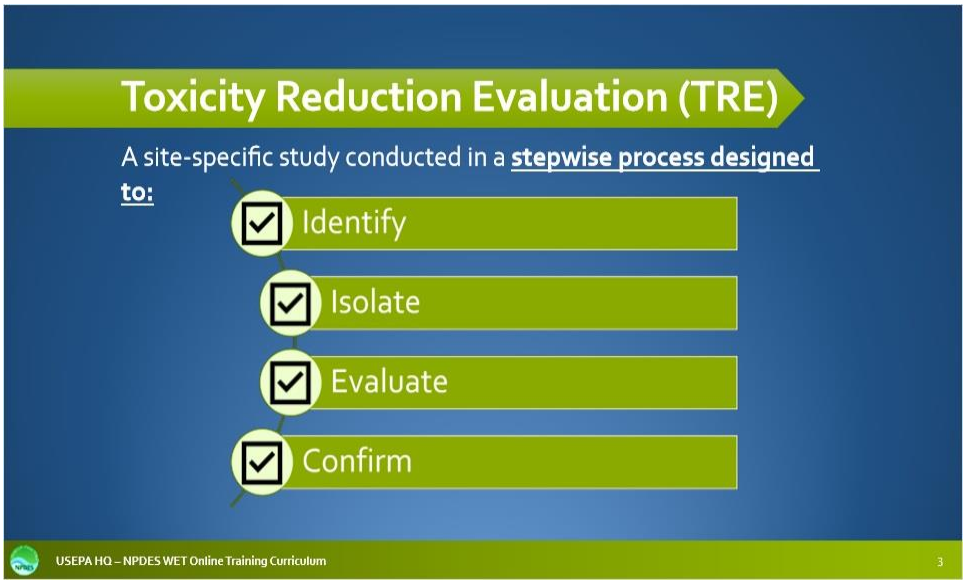
Notes:

First, the introductions.
Your speakers for this presentation are, me, Laura Phillips, USEPA's NPDES WET Coordinator with the Water Permits Division within the Office of Wastewater Management at the USEPA Headquarters in Washington, D.C., and Jerry Diamond, a USEPA Headquarters contractor and an aquatic toxicologist with Tetra Tech, Incorporated in Owings Mills, Maryland. Second, now for those housekeeping items.
You should be aware that all the materials used in this presentation have been reviewed by USEPA staff for technical and programmatic accuracy; however, the views of the speakers are their own and do not necessarily reflect those of the USEPA. The NPDES permits program, which includes the use of WET testing, is governed by the existing requirements of the Clean Water Act and USEPA's NPDES permit implementation regulations. These statutory and regulatory provisions contain legally binding requirements. However, the information in this presentation is not binding. Furthermore, it supplements, and does not modify, existing USEPA policy and guidance on WET in the NPDES permit program. USEPA may revise and/or update the contents of this presentation in the future.
Also, this module was developed based on the live USEPA Headquarters' NPDES WET course that the Water Permits Division of the Office of Wastewater Management has been teaching to USEPA regions and states for several years. This course, where possible, has been developed with both the non-scientist and scientist in mind. Also, while not necessary, basic knowledge of biological principles

and WET will be helpful to the viewer. Prior to this course, a review of the USEPA's NPDES Permit Writers' online course, which is available at USEPA's NPDES website, is recommended.

When appropriate a blue button will appear on a slide to provide access to more information. By clicking this button, additional slides will present information regarding either freshwater or marine USEPA WET test methods. When these additional slides are finished, you will be automatically returned to the module slide where you left off. The blue button on this slide provides the references for USEPA's WET test methods that will be presented throughout this module.

Now that you know who we are and we've covered the housekeeping item, let me turn this over to Jerry to go over Toxicity Reduction Evaluations and Toxicity Identification Evaluations.



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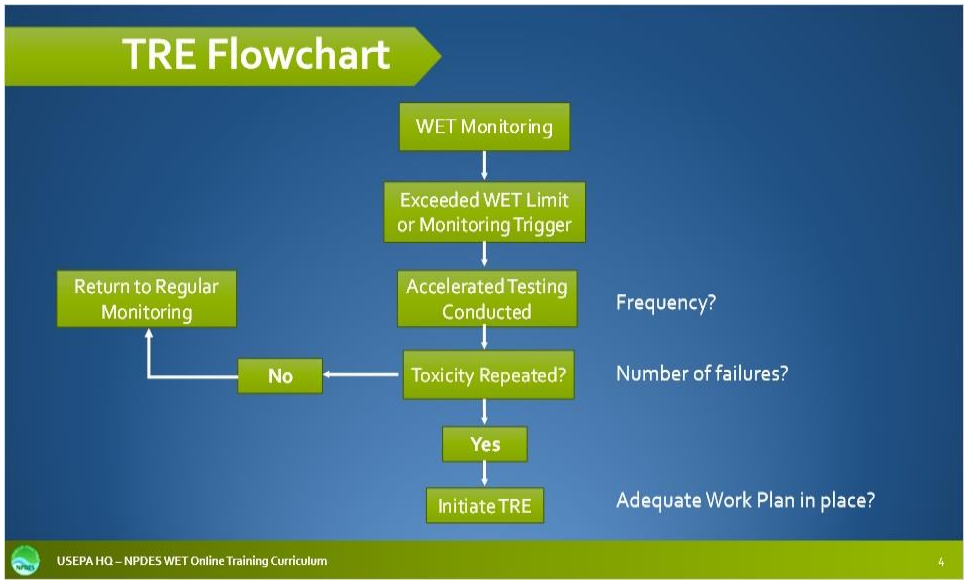
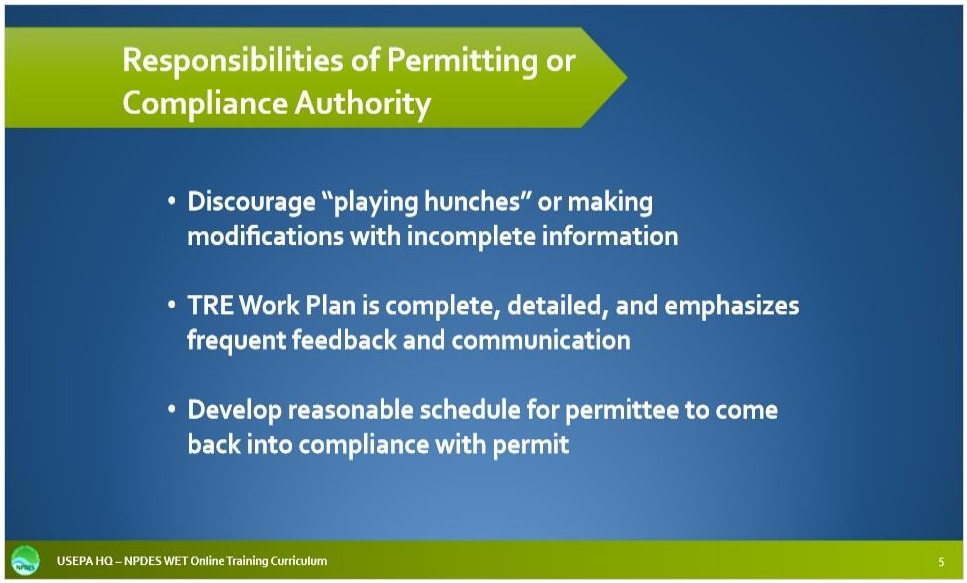
Thanks, Laura! This module reviews the Toxicity Reduction and Toxicity Identification Evaluation process that is used under the USEPA NPDES permit program to enable permittees to identify and reduce toxicity that is observed in Whole Effluent Toxicity, or WET, tests. A toxicity reduction evaluation, or TRE, is a site-specific study of the effluent or wastewater at a treatment facility, conducted in a stepwise process. Once the identification/isolation process has confirmed the potential cause of toxicity, the evaluation step helps determine how to reduce or treat the chemical or chemicals causing toxicity in the effluent. If the evaluation step is completed successfully, the TRE should confirm that the actions chosen to reduce toxicity are successful. There are potentially many ways to reduce toxicity depending on the cause, which will be covered later in the module.



Notes:

Let's take a moment to review how the need for a permittee to conduct a TRE may arise. In the flow chart example illustrated on this slide, the discharger has conducted WET monitoring in accordance with their NPDES permit. During the WET monitoring, the NPDES WET permit limit was exceeded. NPDES WET permit limits are established to prevent excursions of state WET water quality standards, so an exceedance of a WET permit limit can result in permit requirements such as triggers. Permit triggers are actions to be taken by the permittee to identify and resolve the toxicity to come back into compliance with their permit. Therefore, based on WET conditions in the NPDES permit, the permittee is required to conduct accelerated WET testing. Accelerated monitoring requirements can vary from state to state, but there's usually a requirement for more frequent WET testing over a short time period, often just a few weeks, to determine if the toxicity is persistent. If the effluent toxicity is not measured at a level that exceeds the permit limit based on the data generated by the accelerated WET testing, the permit usually allows for a return to the previous WET monitoring frequency schedule. If there is still an exceedance of the WET permit limit based on the accelerated WET testing data, the TRE process is initiated. The right side of the slide highlights important questions regarding certain steps in the process, to emphasize the recommendation that answers these questions which should be clearly described in the permit's WET conditions. The final point of this slide is that it is extremely important to have an adequate work plan that includes a schedule and reporting requirements throughout the process and written into the permit, and particularly before the TRE

is initiated.

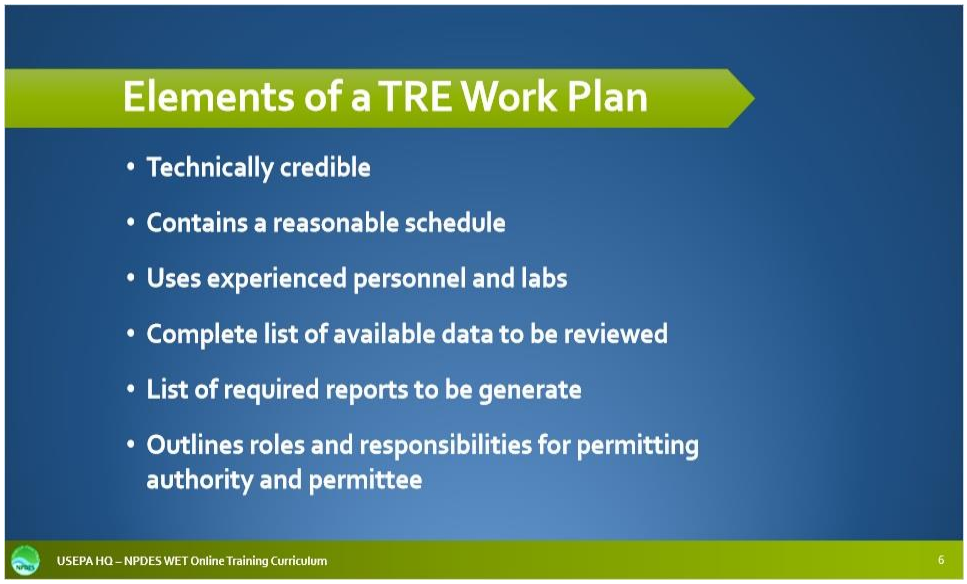


Notes:

It is important to understand that most of the work completed in the TRE is conducted by the permittee through their labs or consultants. However, it is equally important for the USEPA or state NPDES permitting authority to ensure that the TRE process is on track and that the permittee will resolve the toxicity problem in an appropriate and timely manner.

One recommendation is that the NPDES permitting authority discourage the permittee from "playing hunches" or progressing forward with a treatment modification based on improper or incomplete information regarding the cause of toxicity. On occasion, a permittee may believe they have the answer, and may take measures that can not only be costly from a financial standpoint, but also from a time perspective. This is where the NPDES permitting authority can provide key recommendations to the permittee towards ensuring that all available information and possible strategies are considered in the evaluation.

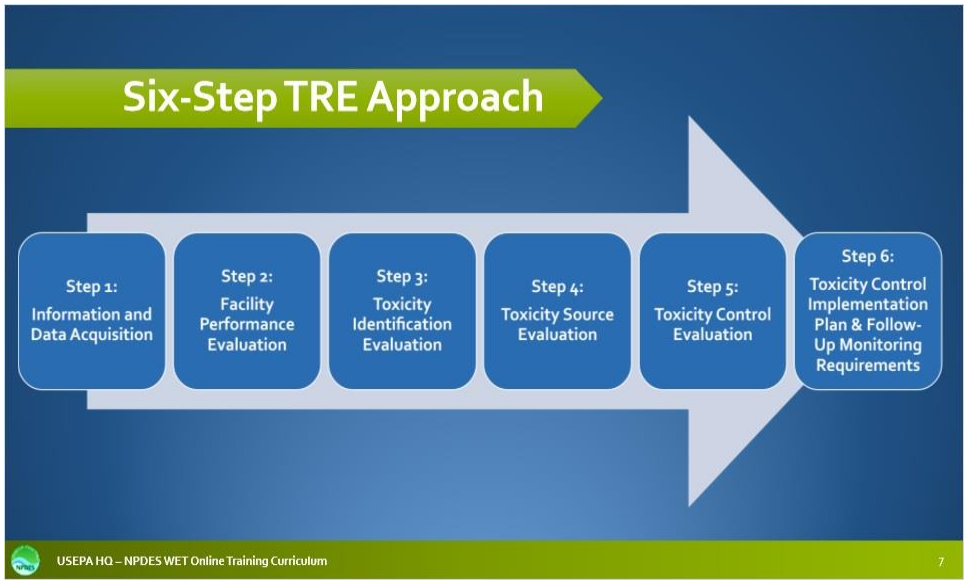
Another important recommendation is that the permittee develop a TRE work plan that is sufficiently detailed and includes frequent communication between the NPDES permitting authority and the permittee. TRE work plan requirements vary from state to state; however, one common feature is that the TRE plan includes a schedule and reporting requirements to ensure that the effluent toxicity is reduced or eliminated to achieve compliance with the permit in the required time frame.



Notes:

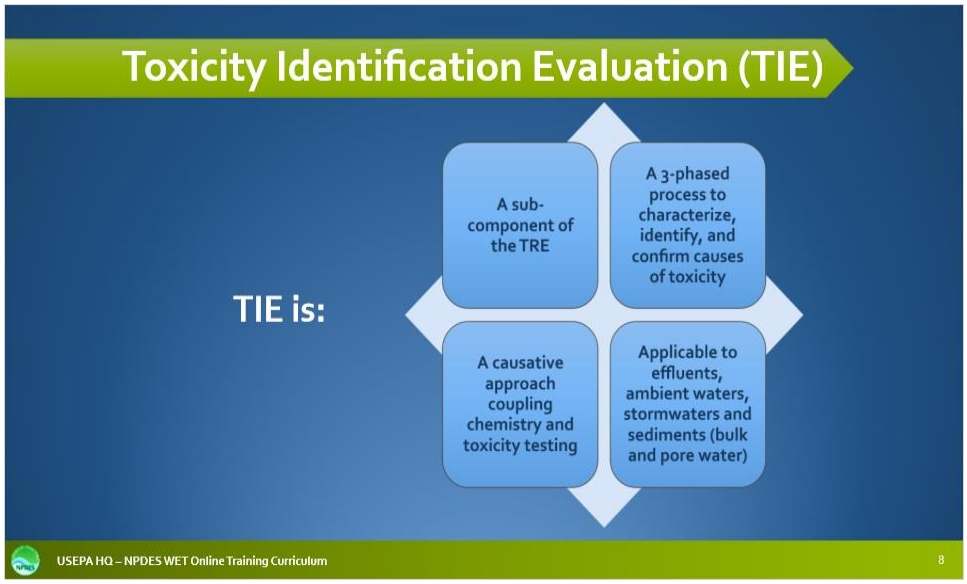
Based on USEPA's experience, a TRE is most likely to be successful if there is a good partnership between the facility, including the influent, and experts on toxicity issues, including how to determine the causes of the effluent toxicity. A TRE often will involve the use of several disciplines, including wastewater treatment engineering, chemistry, process engineering, toxicology, and perhaps hydrology. Therefore, it is important that there are experts on the team to ensure a successful TRE. The more experience TRE team participants have, the better. This is especially true for the toxicologist on the team because they can help link water quality characteristics to toxicity for different USEPA WET test species.

Regardless of the facility, a TRE almost always starts with a review of the available data. Relevant data includes influent and effluent chemical and physiochemical data, facility treatment data, and WET test data, including the physicochemical data collected during the WET tests and the raw toxicity data from the lab. Often, a thorough review of these data can be very useful to help determine what might be causing toxicity in the effluent. Facility treatment information that is often very useful in conjunction with the WET data are parameters such as effluent chemical oxygen demand (COD), biochemical oxygen demand (BOD), mix liquor solids, volatile solids, and removal rates of COD and BOD based on influent and effluent concentrations. The work plan should include the data and other information available for the evaluation, any interim reports or other deliverables to be sent to the NPDES permitting authority, and the roles and responsibilities of the TRE plan's team members.



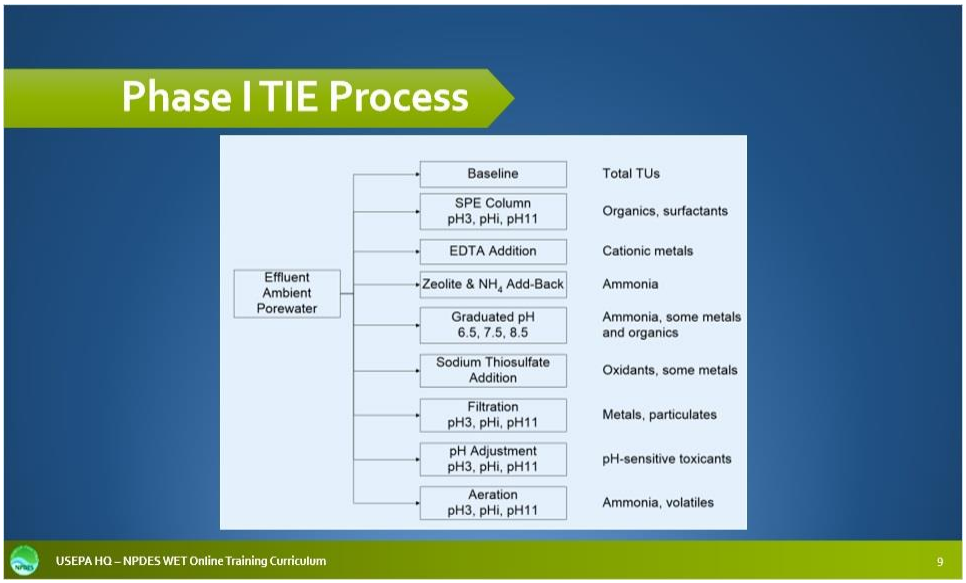
Notes:

A TRE consists of six steps, but not all six steps may be required depending on the facility site-specific situation. The acquisition of relevant information and data, step 1, is a necessary first step in any TRE. Step 2, evaluation of the facility performance, is nearly always needed in a TRE. Step 3 is the toxicity identification evaluation, or TIE, and is optional. There are multiple ways to resolve an effluent toxicity issue, and it may not always be the most expedient strategy to focus first on identifying the exact cause of the toxicity. While knowing the exact cause of toxicity may be optional, the evaluation of the source of toxicity, step 4, is almost always a critical step in the TRE process. The toxicity source evaluation is particularly important where a facility may have multiple sources, such as a municipal wastewater facility or a large industrial facility with multiple waste streams. Step 5, the toxicity control evaluation, is always required within the six-step TRE approach. This step evaluates how the effluent toxicity will be controlled based on either an identification of the toxicant(s) or the source of toxicity. Finally, in step 6, the toxicity control implementation plan and follow-up WET and/or chemical monitoring are incorporated into the TRE plan to confirm that effluent toxicity is controlled and there is permit compliance.



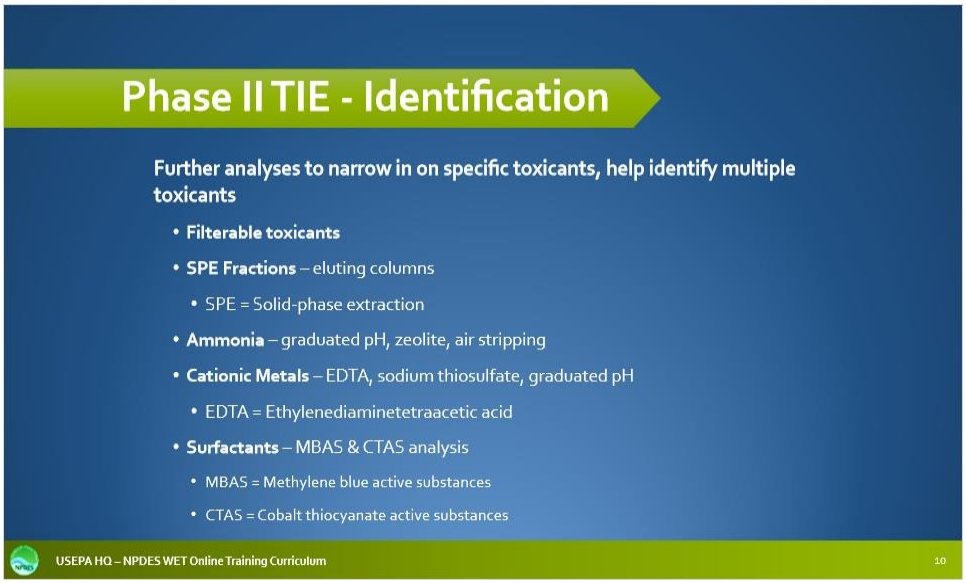
Notes:

As noted on the previous slide, one optional step in the TRE approach is to identify the exact cause of effluent toxicity. This is commonly referred to as a TIE. Although not necessary, a TIE can often be very helpful in a TRE, because toxicity can be more certainly controlled if the identity of the toxicant(s) is known. The TIE is a three-phase process that characterizes, identifies, and confirms the cause or causes of toxicity. A TIE couples effluent chemical analysis and WET test results. Although it may take some effort to identify the exact cause of effluent toxicity, particularly in a very complex effluent situation, using experienced WET testing laboratories and consultants can help ensure that the TIE is not an expensive, time-consuming venture. TIEs are applicable to evaluating toxicity of permitted effluents, ambient waters, storm waters and sediments, including bulk sediment or pore waters.



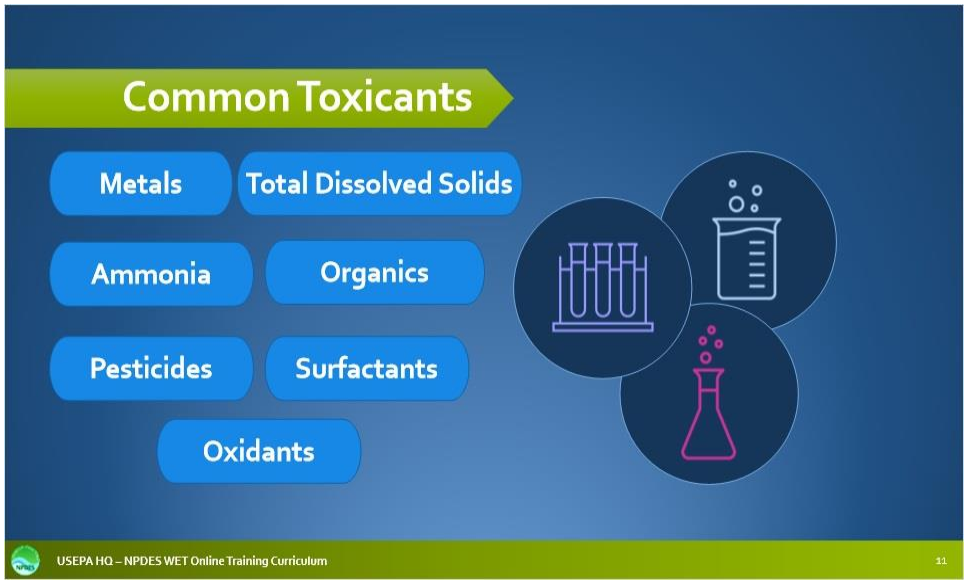
Notes:

The TIE flow chart illustrated on this slide is from the USEPA Phase I TIE guidance manual. Although this guidance was published in the early 1990s, it is still very relevant. Also, many labs have added to the options presented in the TIE Phase I document based on an increasing array of specialized columns and other types of treatments that are specific for certain chemicals.



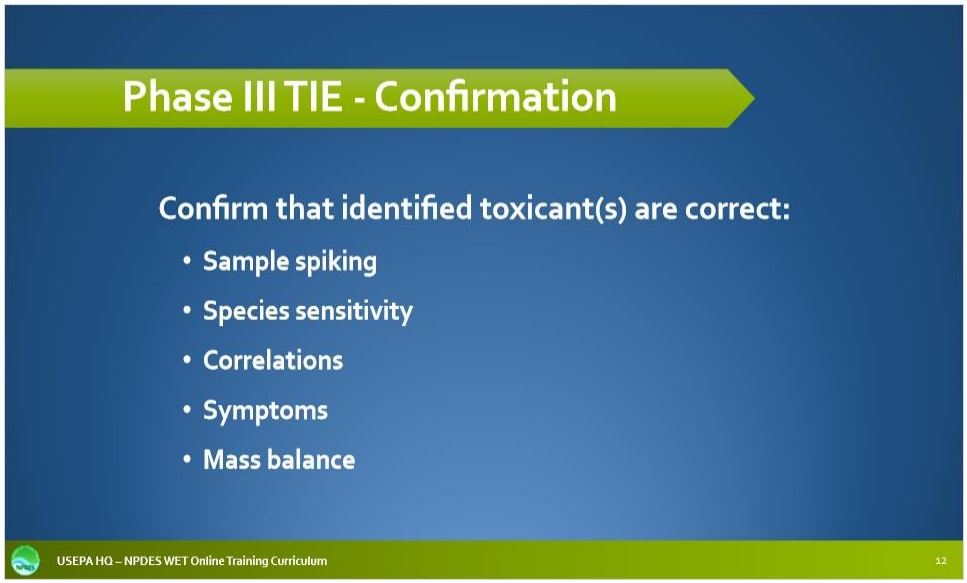
Notes:

After conducting Phase I of the TIE, the types of chemical that may be causing toxicity should be identified, for example, metal, non-polar organic, oxidant, etc. Phase II of the TIE process attempts to identify the specific chemical or chemicals causing the toxicity. The identification of the chemical(s) is accomplished using in-depth analyses that often require more chemical analyses of the effluent and working with certain effluent fractions based on the Phase I TIE results. For example, if air-stripping reduced the toxicity in the TIE Phase I, then ammonia may be responsible for the toxicity, because ammonia tends to volatilize when using air-stripping. TIE Phase II analyses might then use additional treatments to determine whether ammonia is indeed the cause of the toxicity.



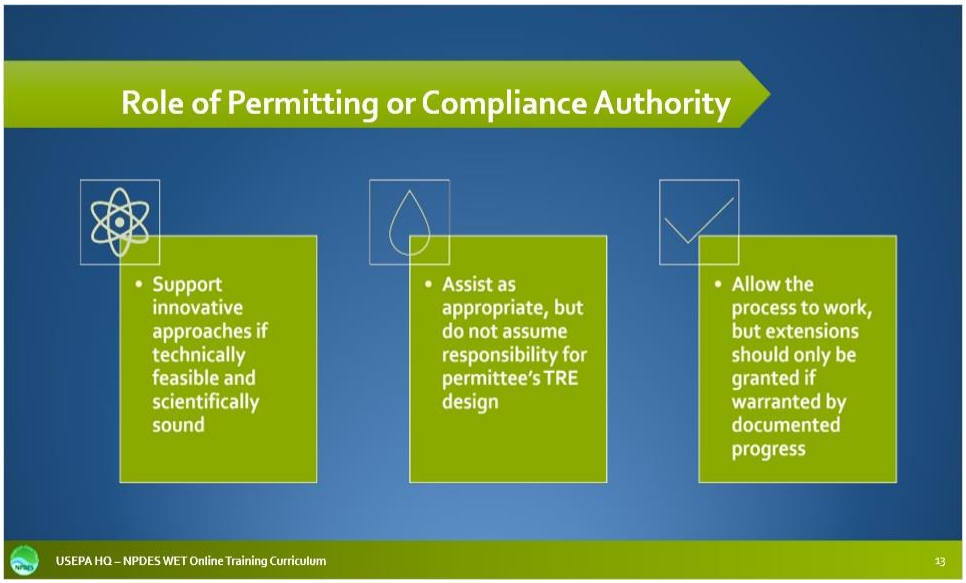
Notes:

Here we list a few of the common toxicants that may be identified using a TIE or when conducting a TRE. Some common toxicants include metals, total dissolved solids, ammonia, organics, surfactants, pesticides, and oxidants. For each of these common toxicants, more details can be found by clicking on the appropriate blue button. When you are done viewing the additional materials you will be returned here to continue with this module.



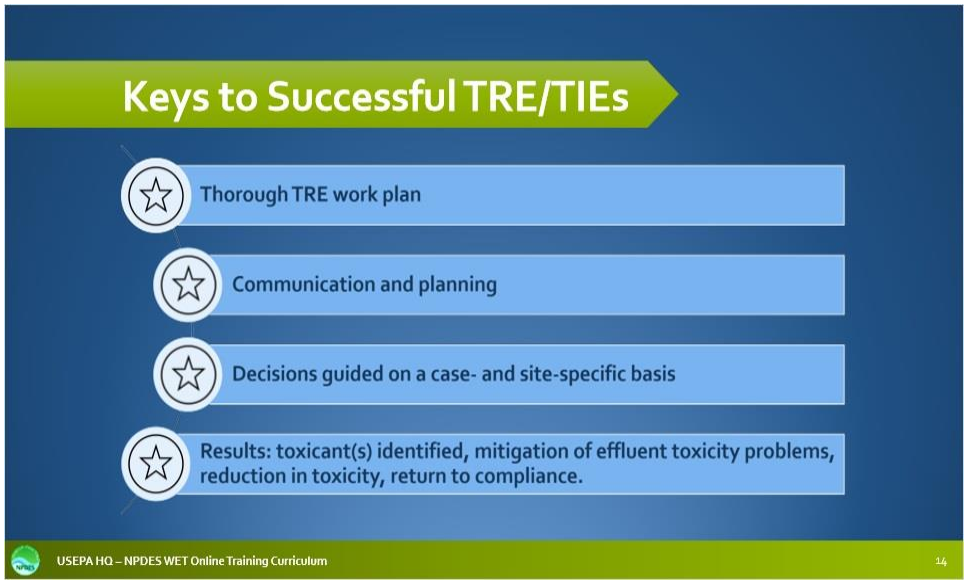
Notes:

In Phase III of the TIE, we confirm what we believe is the cause of the toxicity as determined in Phase II. In Phase III, different analyses including some statistical analyses may be conducted to confirm the cause of the toxicity. Phase III of a TIE may use the following to confirm the cause of toxicity identified during Phase II of the TIE: sample spiking, species sensitivity, correlations, symptoms, and mass balance. At this point, there may or may not be a need for more chemical analyses or toxicity testing. Other lines of evidence such as more in-depth treatability information may be used in Phase III to confirm Phase II conclusions. USEPA has Phase II and Phase III TIE guidance manuals and the TIE procedures listed here are discussed in more detail in the USEPA TIE manuals.



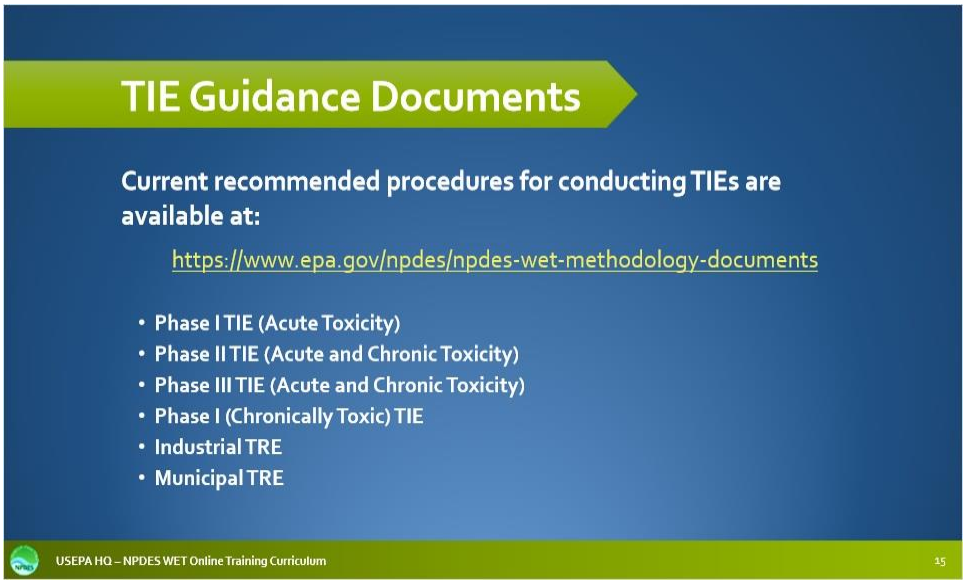
Notes:

As previously mentioned, the role of the NPDES permitting authority in TIEs is to support innovative approaches that are technically feasible as well as scientifically sound, and to discourage approaches that are not results-oriented, are costly, or require too much time to resolve the toxicity. In some instances, the permittee may need to use novel approaches to identify the cause of toxicity. The NPDES permitting authority can assist the permittee by providing technical information, where appropriate. However, conducting the TIE/TRE is the responsibility of the permittee, not the NPDES permitting authority. The role of the NPDES permitting authority is to allow the TIE/TRE process to proceed and to confirm that the permittee is making good progress towards completing the TRE.



Notes:

TRE/TIE success is based on proper planning and communication. One key to a successful TRE/TIE is developing a thorough TRE work plan, then frequently re-examining and updating it as new information is obtained. By discouraging making decisions based on instinct, intuition, or an educated guess, the potential for a successful TRE/TIE increases. Communication between permittees and the permitting authority is also key since both parties have a vested interest in resolving the toxicity issue quickly. Decisions should be communicated by both parties and should help guide the TRE on a case- or site-specific basis. The goal is not always to identify the toxicant, but to mitigate the effluent toxicity and return to compliance with the permit.

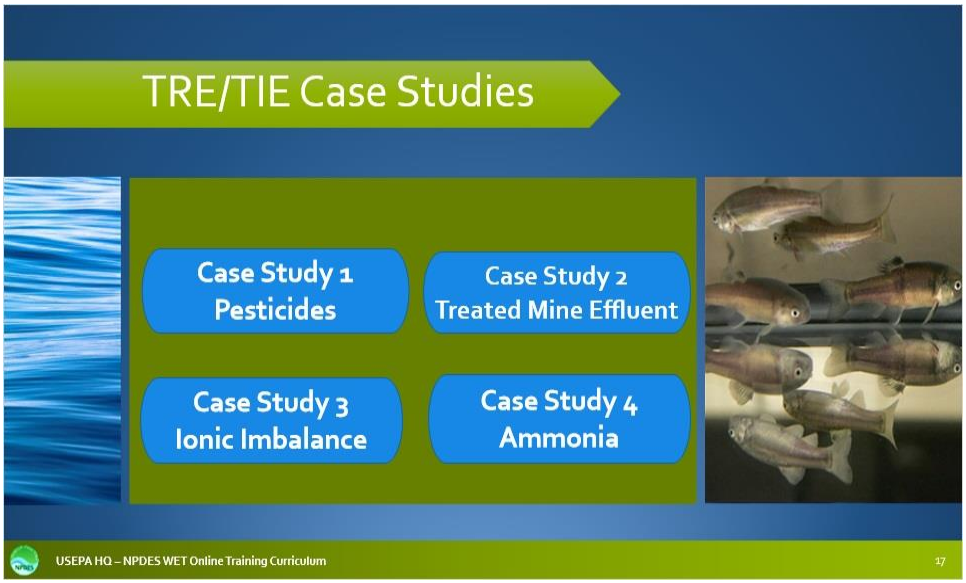


Notes:

USEPA TIE guidance documents are available on the USEPA Office of Wastewater Management's NPDES website. These are the current recommended procedures for conducting TREs/TIEs. On the website, you will find a guidance document for each of the three phases of TIEs, a Phase I TIE guidance document for chronically toxic effluents, and the guidance documents for conducting toxicity reduction evaluations for industrial and municipal effluents.

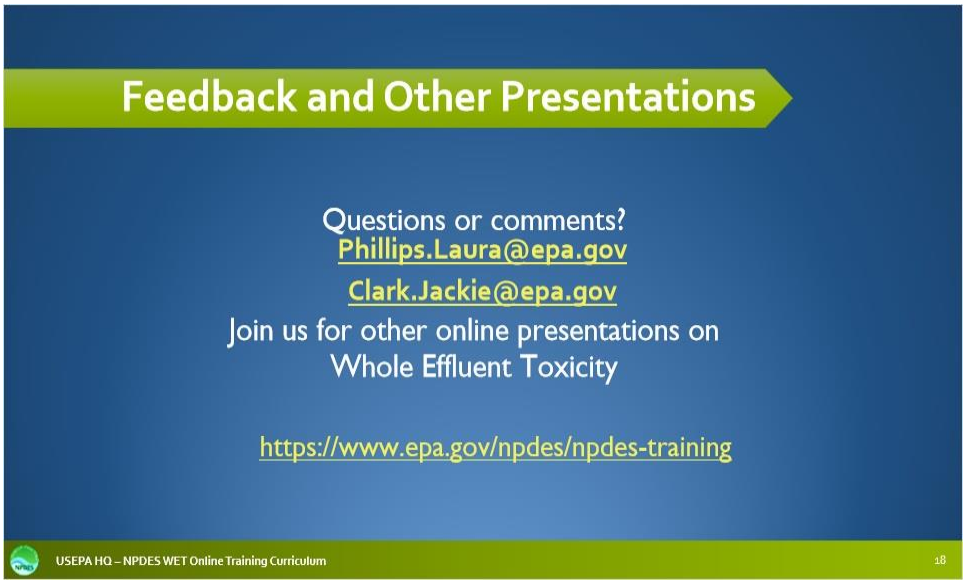
Notes:

We want to remind you that the TRE work plan is critical and should be technically credible, contain a reasonable schedule, and use experienced personnel and laboratories. The TRE work plan should also provide for ongoing re-evaluations of the plan as necessary. The work plan should encourage decisions that are guided by the site-specific situation and emphasize early and frequent communication between the permittee and the NPDES permitting authority. The focus of the TRE work plan should be on mitigating the effluent toxicity problems, reducing, abating or eliminating the toxicity, and returning to compliance with the permit as quickly as possible. A high level of QA/QC during the TIE and TRE process is essential for ensuring that the results aid in finding a solution to remove the effluent toxicity and return to full NPDES permit compliance. Finally, as the TIE and TRE process can be very challenging, the NPDES permitting and/or compliance authority should offer some flexibility for the permittee to alter the approach when appropriate and technically necessary.



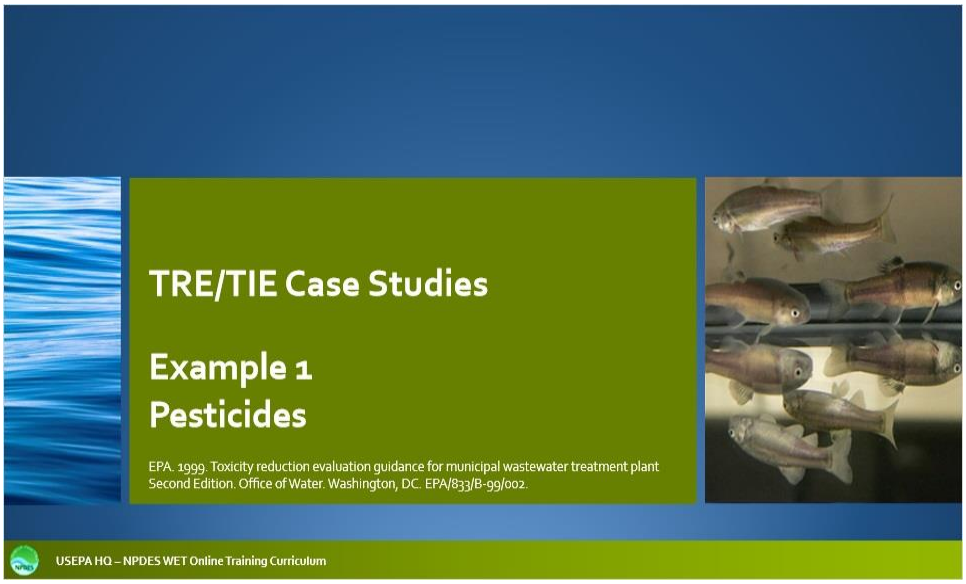
Notes:

Now let's examine a few case study examples where the TIE and TRE process was used to successfully diagnose and address the cause of toxicity so the permittee would be back in compliance with the NPDES permit.



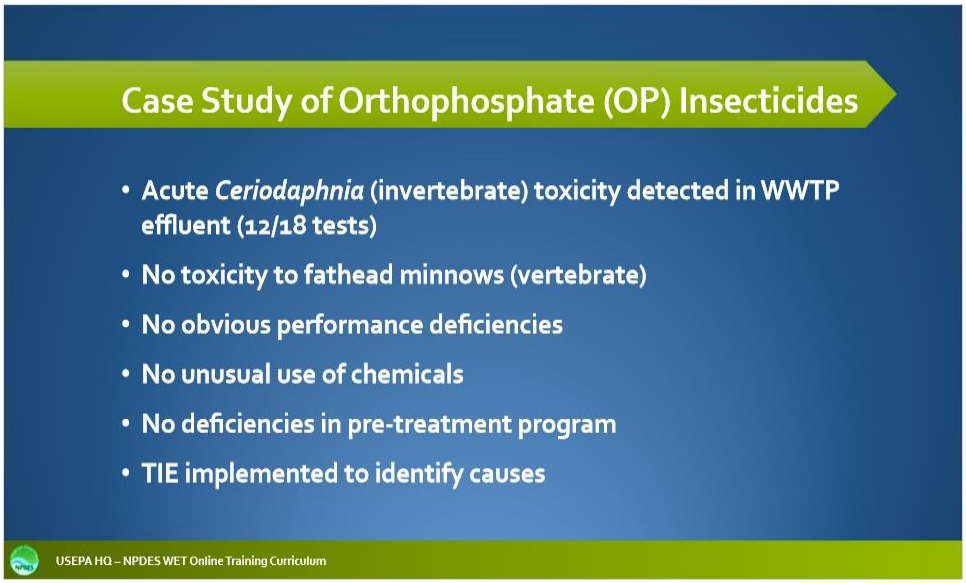
Notes:

Thank you for joining us for this USEPA's NPDES Whole Effluent Toxicity training presentation. We hope that you have enjoyed it!
If you have any questions or comments on this or any part of the USEPA's NPDES WET online training curriculum, click on the email address given on this slide to send a message to Laura Phillips or Jackie Clark, USEPA HQ NPDES WET Coordinators.
Remember, you will find all of the USEPA's NPDES WET online training presentations, under the USEPA's NPDES training section found on the Office of Wastewater Management's NPDES website.
See you next time!



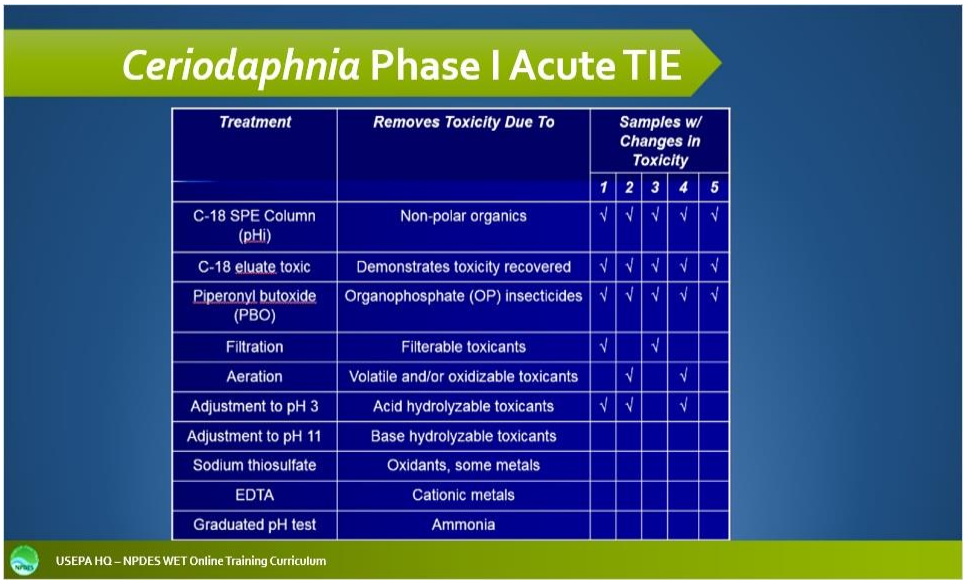
Notes:

In this first example, we will look at a municipal effluent that had a toxicity issue involving commonly used pesticides.



Notes:

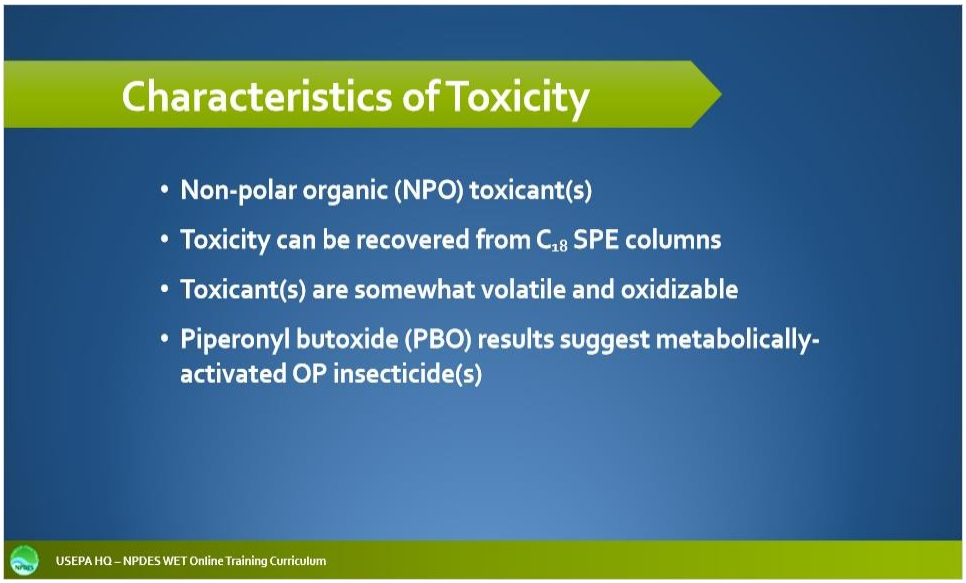
In this example, the effluent of a wastewater treatment plant, or WWTP, exhibited acute toxicity to *Ceriodaphnia dubia*, an invertebrate water flea, in 12 of 18 acute toxicity tests. Also, there was no observed toxicity to the tested fish, fathead minnows. As part of the TIE, the facility's treatment performance was reviewed, and no obvious performance deficiencies were noted. Furthermore, the facility met all the other water quality-based effluent limits in their NPDES permit. There had been no unusual use of new or different treatment chemicals, and no deficiencies were noted in their pre-treatment program. The wastewater treatment plant therefore initiated a TIE.



Treatment	Removes Toxicity Due To	Samples w/ Changes in Toxicity				
		1	2	3	4	5
C-18 SPE Column (pHi)	Non-polar organics	√	√	√	√	√
C-18 eluate toxic	Demonstrates toxicity recovered	√	√	√	√	√
Piperonyl butoxide (PBO)	Organophosphate (OP) insecticides	√	√	√	√	√
Filtration	Filterable toxicants	√		√		
Aeration	Volatile and/or oxidizable toxicants		√		√	
Adjustment to pH 3	Acid hydrolyzable toxicants	√	√		√	
Adjustment to pH 11	Base hydrolyzable toxicants					
Sodium thiosulfate	Oxidants, some metals					
EDTA	Cationic metals					
Graduated pH test	Ammonia					

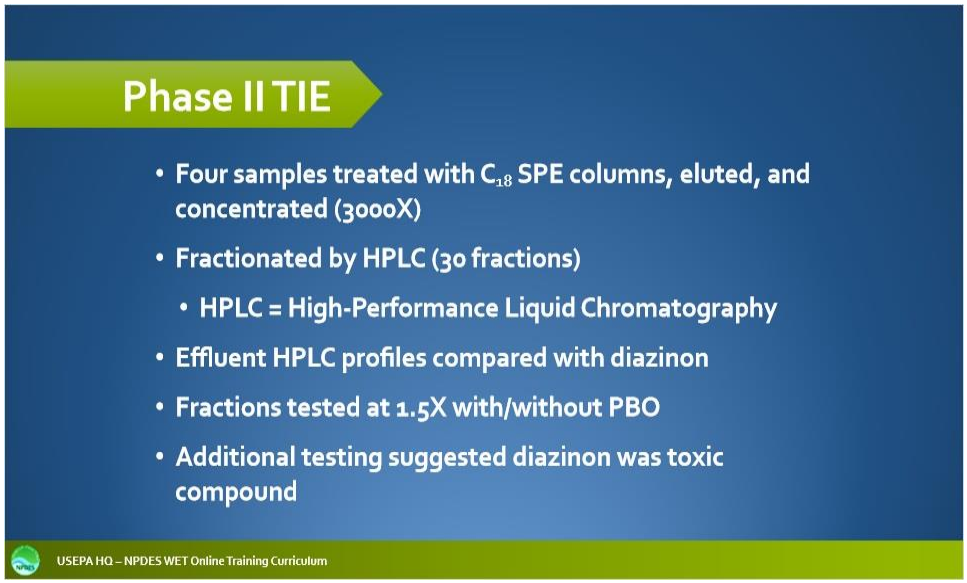
Notes:

This table shows the results of the Phase I TIE WET tests using acute exposures to *Ceriodaphnia dubia*. The first column shows the treatments used on sub-samples of the effluent, many of which were presented in this module. One additional treatment, piperonyl butoxide (PBO), was used in this case. PBO specifically eliminates toxicity associated with organophosphate insecticides. The numbered columns refer to five different effluent samples collected over time that were assessed using USEPA WET tests. Testing multiple effluent samples helps ensure that the cause or causes of toxicity is adequately characterized. The checkmarks in each column indicate whether, for that sample, the treatment was effective at providing information about toxicity. In most of the cases, the checkmark means that the treatment reduced effluent toxicity. Treatments that did not provide any information or a reduction in toxicity do not have a checkmark.



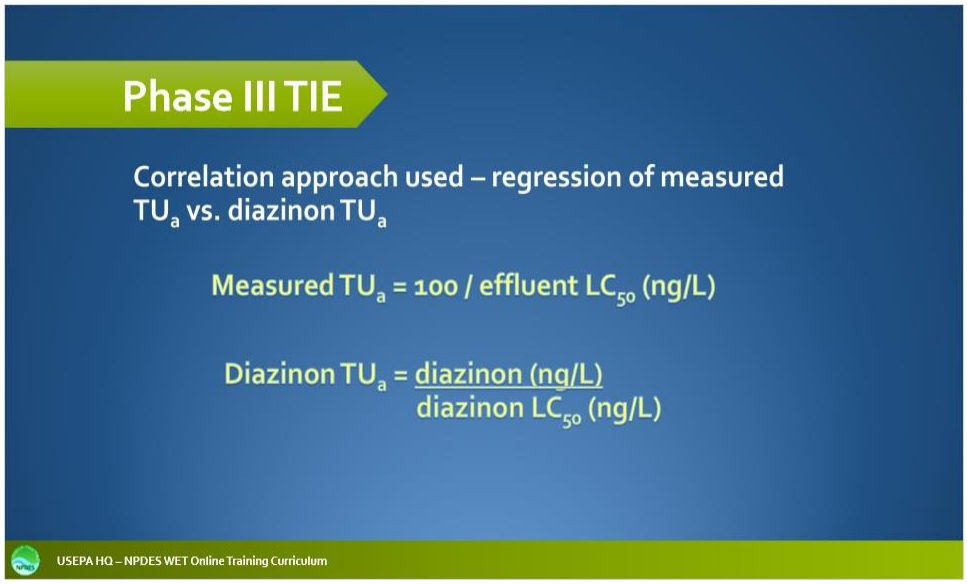
Notes:

Looking at the results in the table on the previous slide, the C_{18} column reduced the toxicity, indicating that the toxicant could be a non-polar organic. Further testing using the SPE column demonstrated that toxicity could be recovered, further implicating a non-polar organic chemical as the cause. Other treatments used, including aerating the sample and lowering the pH to 3 before a return to the initial effluent pH, indicated that the toxicant or toxicants are somewhat volatile and oxidizable. The elimination of toxicity in samples treated with PBO is very specific, suggesting a metabolically-activated organophosphate insecticide may be responsible for the observed toxicity.



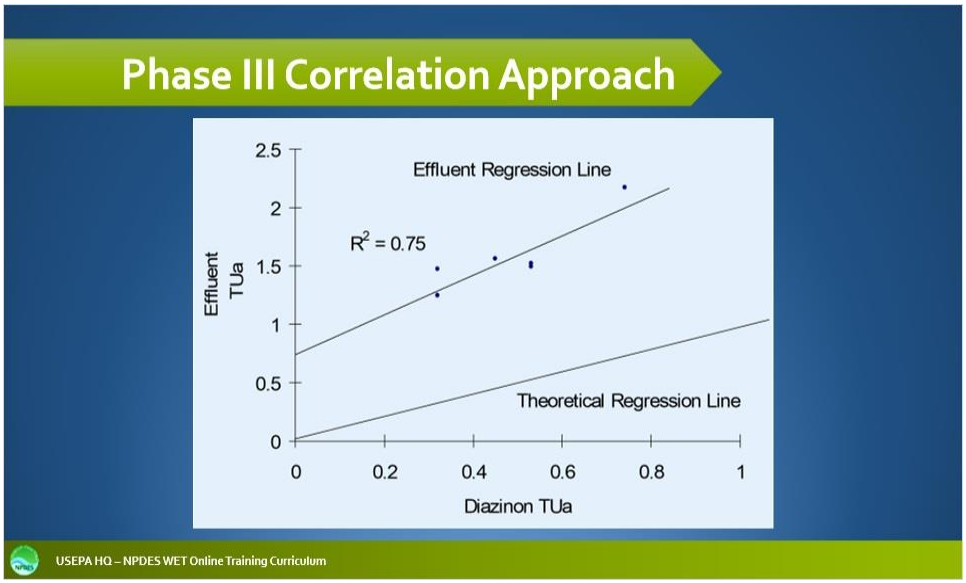
Notes:

During Phase II of the TIE, chemical analyses, such as high-performance liquid chromatography, or HPLC, were conducted to identify the chemical toxicant. Using HPLC, the organophosphate insecticide, diazinon, was identified. Interestingly, diazinon was not one of the chemicals being monitored by this facility in their NPDES permit. The facility had no knowledge that this chemical could be entering their plant, perhaps through storm-water runoff from residential lawns or from commercial users of diazinon, who may have discarded it down sink drains.



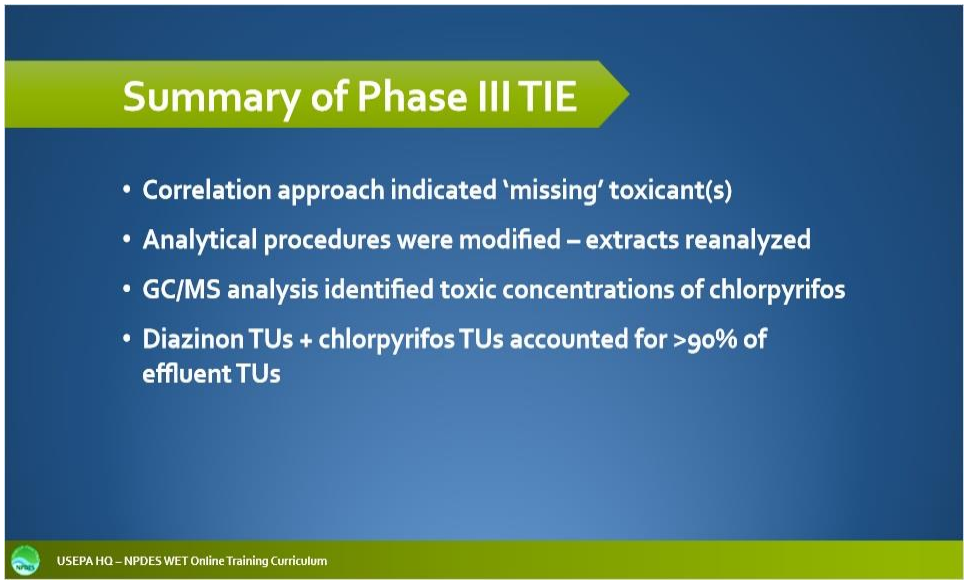
Notes:

Phase III of the TIE process is the confirmation of the cause of the toxicity. To confirm that diazinon was the cause of effluent toxicity, the correlation between effluent diazinon concentrations over time and the associated effluent toxicity results using acute *Ceriodaphnia* WET testing was conducted. To do the comparison, both the diazinon effluent concentrations and the WET data expressed as *Ceriodaphnia* LC_{50}s were converted to Toxicity Units, or TUs. For acute toxicity, the TUs are calculated as 100 divided by the observed LC_{50}. For diazinon, the TUs were calculated by dividing diazinon concentration in the effluent by the known diazinon acute *Ceriodaphnia* LC_{50} data. By converting the data to TUs, two expressions of TUs can be directly compared. If diazinon was the sole cause of toxicity, there should be approximately a 1:1 relationship between the two types of TUs.



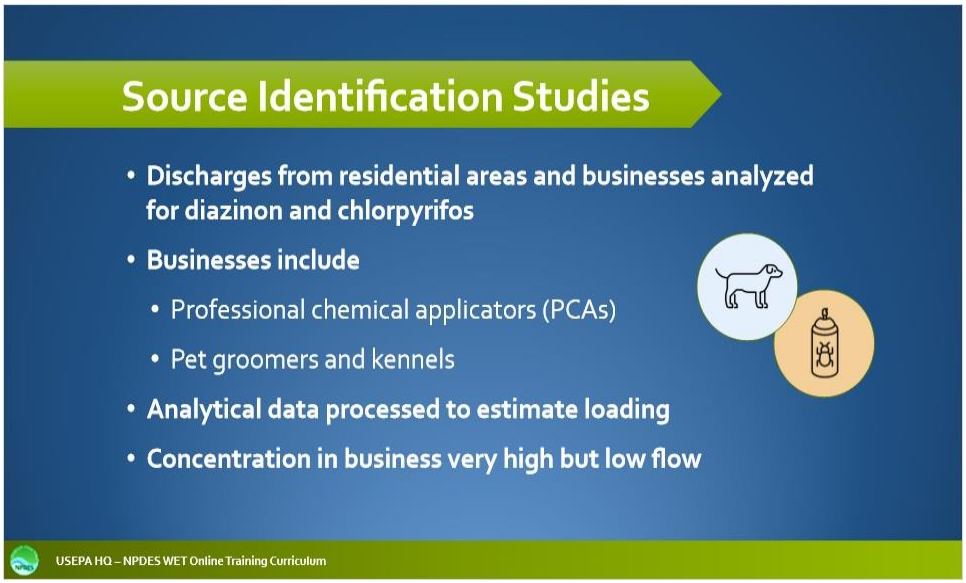
Notes:

During Phase III of the TIE, a correlation approach was used by plotting the effluent acute TUs on the y-axis and diazinon acute TUs on the x-axis. What this graph indicates is that there is not a 1:1 relationship between the two forms of TUs and, in fact, there was more effluent toxicity than would be expected based on the known diazinon acute toxicity to *Ceriodaphnia.* For example, at only half the diazinon concentration that should cause acute toxicity to this WET test species (0.5 TU_a on the x-axis), they observed 1.5 TU_as based on effluent toxicity testing (or an LC_{50}=30-40% effluent). This WET test result indicates that there must be another chemical causing the toxicity in addition to the diazinon.



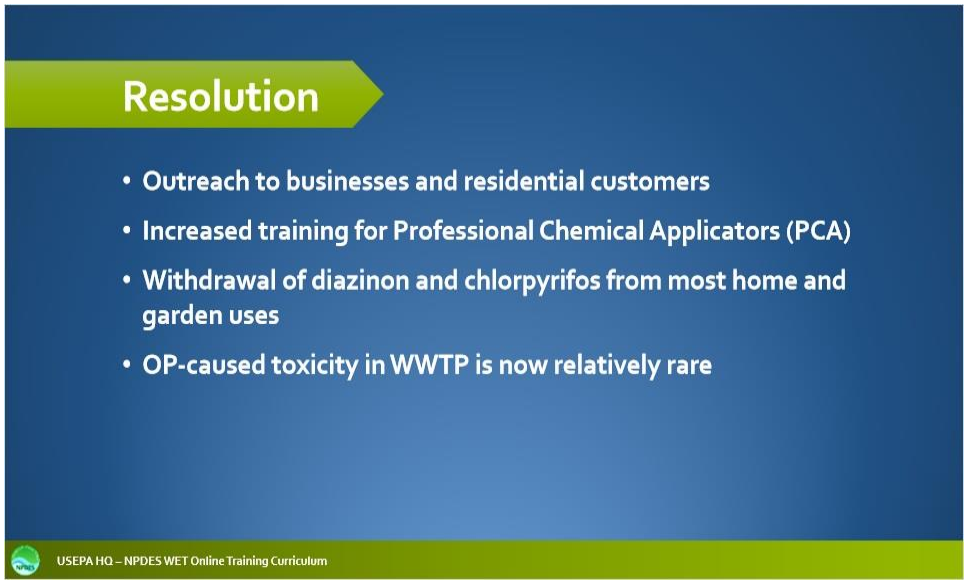
Notes:

The TIE team went back to the lab and did more sophisticated chemical analyses and found that the effluent contained chlorpyrifos, another organophosphate insecticide, in addition to the diazinon. The TUs for diazinon and chlorpyrifos were combined and an almost perfect 1:1 relationship with acute toxicity results was observed. Chlorpyrifos and diazinon are organophosphate insecticides that respond the same way in Phase I TIE testing using PBO. By combining effects of both insecticides, the TIE team obtained a more accurate picture of the causes of effluent toxicity.



Notes:

Now knowing the causes of effluent toxicity, the next task was to determine the source or sources of the two insecticides. The team quickly confirmed that the major sources of these pesticides were residential areas and businesses, particularly those that dealt with pet grooming and similar types of activities where products containing these pesticides were used. So, what did the team do to resolve the toxicity issue?

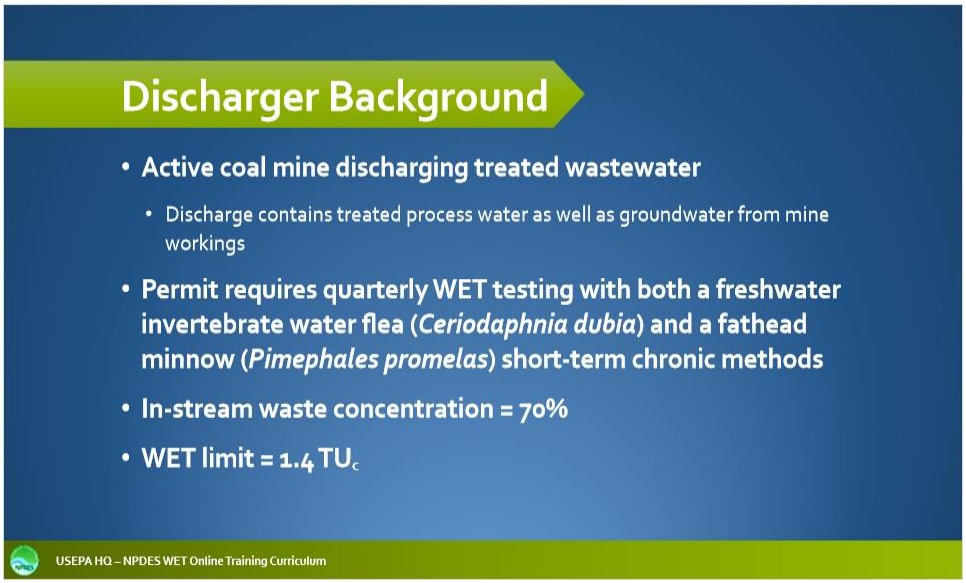


Notes:

The WWTP was already using advanced tertiary treatment. Therefore, the toxicity issue was resolved by finding ways to reduce pesticide loading to the WWTP. The city implemented an intensive public communication and outreach plan to promote the use of safer replacement chemicals and reduce the usage and disposal of these insecticides. The public outreach program was a success and the WWTP came back into compliance with their WET NPDES permit limits. As a result of this TIE and others like it in the state, the state agency developed water quality criteria for these pesticides. These water quality criteria are now used in the NPDES program in effluent monitoring for certain types of facilities and to where appropriate generate permit limits.

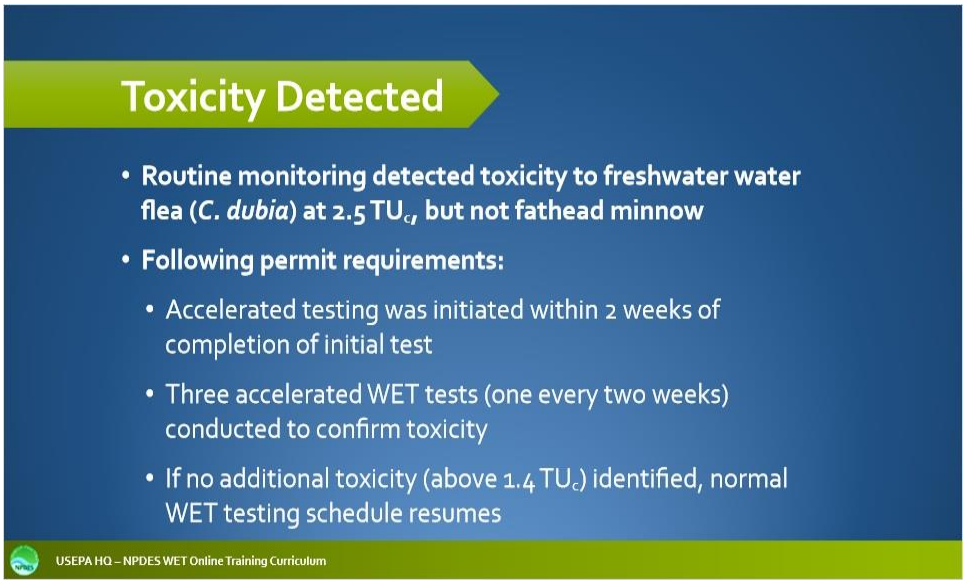
Notes:

In this second example, we will look at an industrial effluent from a mine that had a toxicity issue involving total dissolved solids.



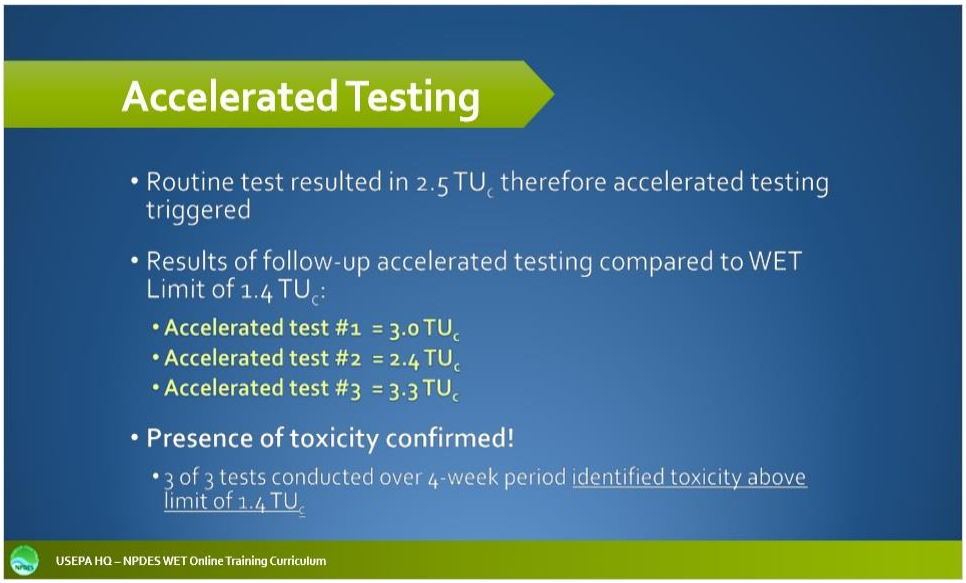
Notes:

In this second TRE example, the industrial effluent discharge from a coal mine contained process water and groundwater from mine workings. The NPDES permit required quarterly chronic WET testing with both a freshwater invertebrate water flea, *Ceriodaphnia dubia,* and fathead minnows, *Pimephales promelas*. The in-stream waste concentration was fairly high at 70%, indicating little effluent dilution was available in the receiving waterbody. The WET limit for this facility was 1.4 chronic toxicity units, or TUs.



Notes:

Toxicity was observed at levels exceeding the facility's WET limit of 1.4 TU_c. Therefore, in accordance with the NPDES permit, accelerated WET testing was conducted to determine whether the toxicity was persistent, which can be helpful when trying to identify the cause of toxicity.

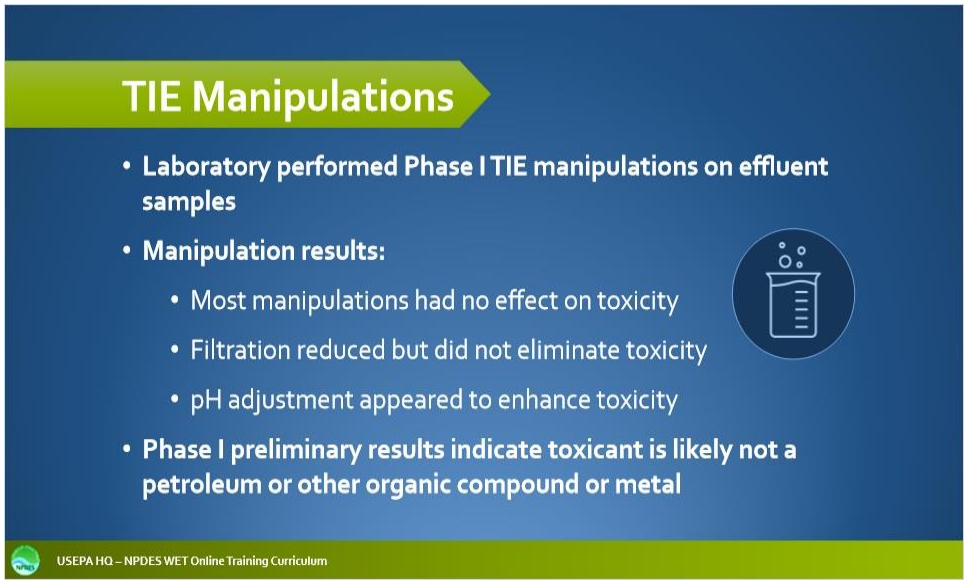


Notes:

The results of accelerated WET testing indicated persistent toxicity in this effluent, confirming toxicity. As a result, the permittee entered into a TRE to determine the potential cause of toxicity and how to reduce it to get back in compliance with the permit limits.

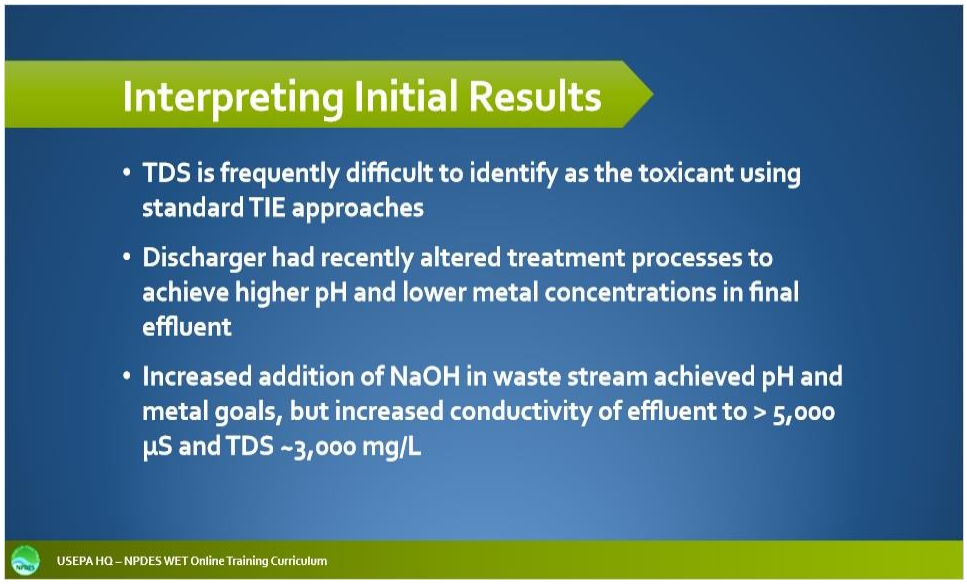
Notes:

Based on the results of the accelerated WET testing, this industrial facility initiated a TIE in accordance with their NPDES permit. As a first step, water quality data for the effluent and the wastewater treatment process data were reviewed. Several potentially toxic chemicals were identified through this review, including petroleum by products, lubricants, organic compounds and surfactants used in the mine, as well as certain metals and total dissolved solids, TDS. TDS, mostly in the form of sodium chloride, was enhanced in this effluent, because sodium hydroxide was used as a treatment chemical to meet the state's pH water quality standards.



Notes:

Following USEPA's Phase I TIE guidance, several treatments were used. However, most of the treatments did not reduce the toxicity of the effluent. Filtration helped, but it did not eliminate the toxicity, and pH adjustments appeared to increase the effluent's toxicity. The TIE Phase I results suggested that petroleum-related chemicals, such as polycyclic aromatic hydrocarbons, or PAHs, as well as metals, were unlikely causes of toxicity, because the respective treatments for these compounds did not decrease effluent toxicity.

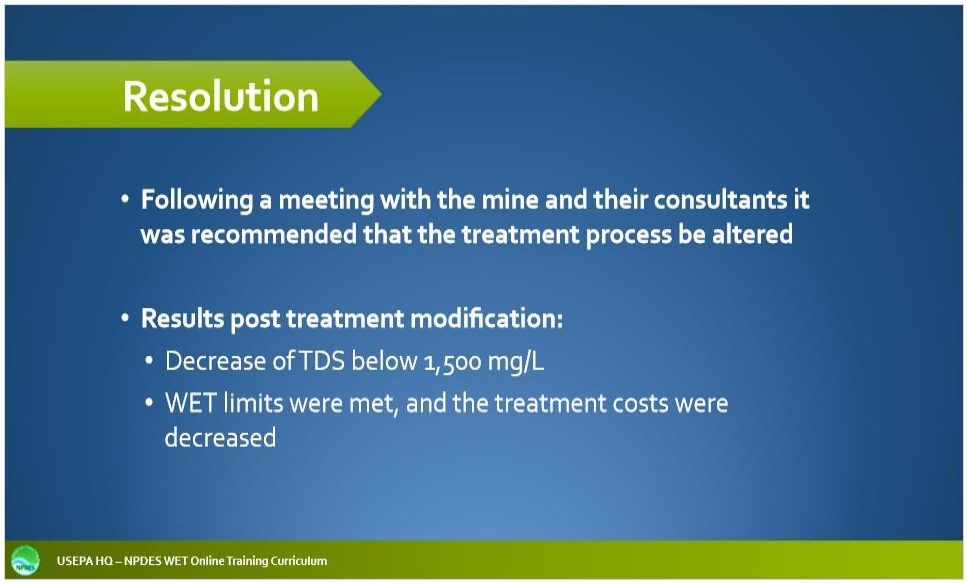


Notes:

The results of the TIE Phase I suggested TDS could be a cause of toxicity. TDS may be comprised of many types of ions depending on the source, which may result in different levels of toxicity depending on the dominant ions present and/or the relative concentrations of the different ions. Therefore, TDS is difficult to confirm as a toxicant using the standard TIE approach, because TDS could be the product of ions (salts) that are not readily removed using the standard TIE treatments. Furthermore, any treatment that removes ions, such as special membrane filtration techniques, are not selective to ions and will remove many other types of chemicals as well. Another piece of useful information, in this example, is the permittee recently altered the treatment by adding more sodium hydroxide, or NaOH, which increased the pH, thereby lowering the metal concentrations in the final effluent to meet the metal effluent limits in the permit. However, adding more sodium hydroxide only increased the concentration of ions as indicated by increased effluent conductivity, which made the effluent even more toxic. The TIE Phase II analyses supported TDS as the cause of toxicity based on the conductivity and toxicity data for *Ceriodaphnia dubia* in chronic WET tests.

Notes:

To confirm that TDS was the cause of toxicity, a mock effluent was prepared using known clean deionized water and salts to mimic the ion concentrations in the effluent. The mock effluent was determined to be chronically toxic to *Ceriodaphnia* in a very similar way as the actual effluent. This alternate TIE research approach helped confirm that TDS was the likely cause of effluent toxicity.

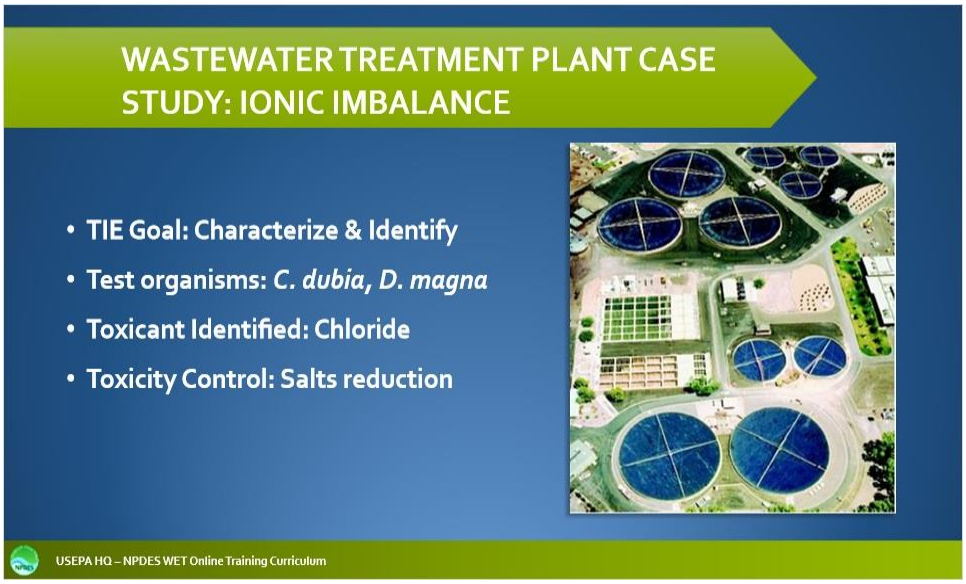


Notes:

Based on the TIE and TRE results, an alternative treatment process was recommended that would reduce the concentration of ions, chloride in particular, in the effluent, but still meet the NPDES permit water quality-based effluent limits. As a result of this alternative treatment, the industrial coal facility was able to reduce its TDS to levels such that the permittee was able to meet their NPDES permit chronic WET limit and decrease their treatment costs since less sodium hydroxide was needed for their treatment process. Hence, in this case, the TIE/TRE process benefited the permittee by lowering the facility's production costs.

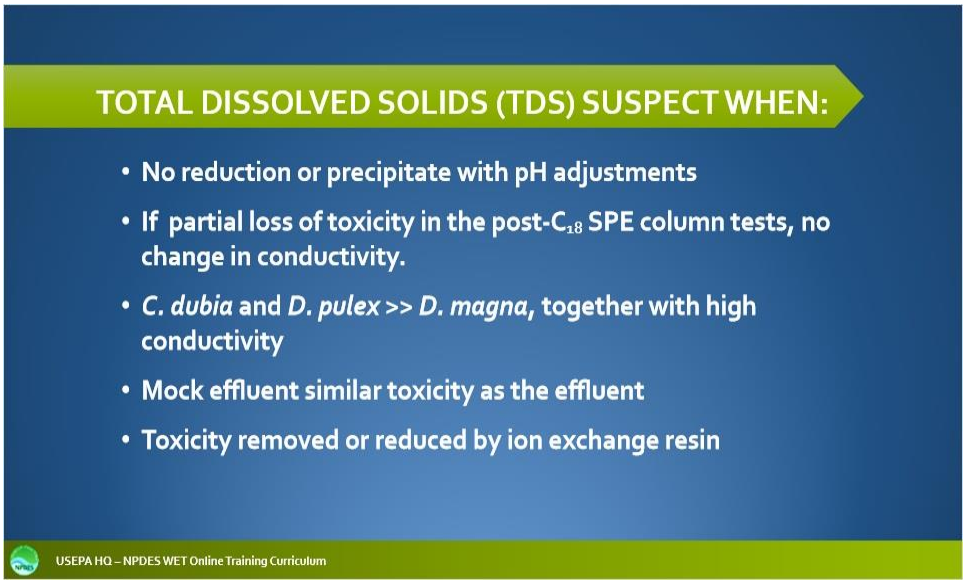
Notes:

In this example, we will look at a municipal effluent that had a toxicity issue involving total dissolved solids and ionic imbalance.



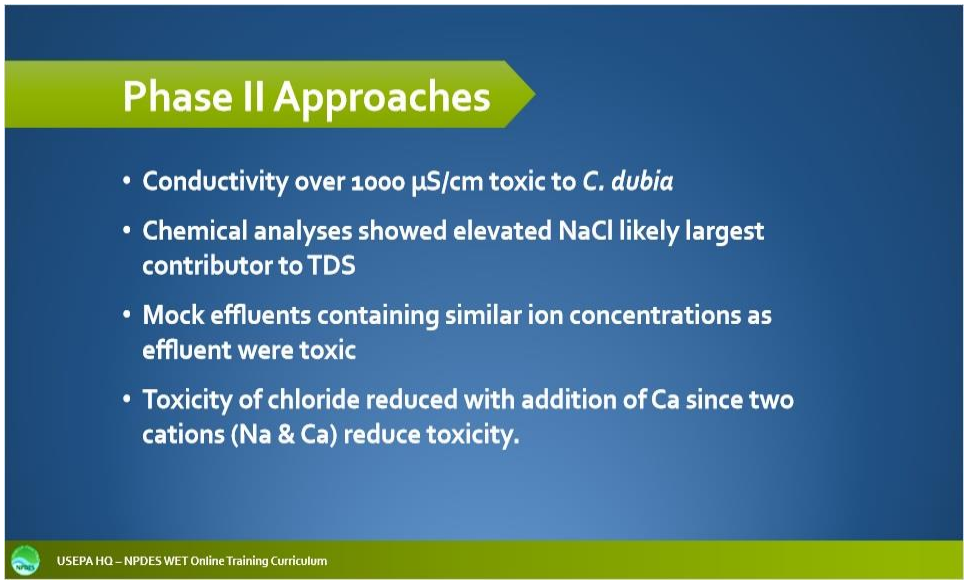
Notes:

In this case study, the municipal WWTP conducted a TIE as part of the TRE process in hopes of identifying the cause of observed effluent toxicity. Toxicity was observed intermittently over multiple years during the permit and was typically associated with invertebrate testing only, including *Ceriodaphnia dubia* and less so with *Daphnia magna*. By conducting a Phase I TIE, the discharger and their laboratory had to move to more specific testing typical of Phase II, as described in the next slide, to aid in identifying the cause of toxicity and developing a toxicity reduction plan.



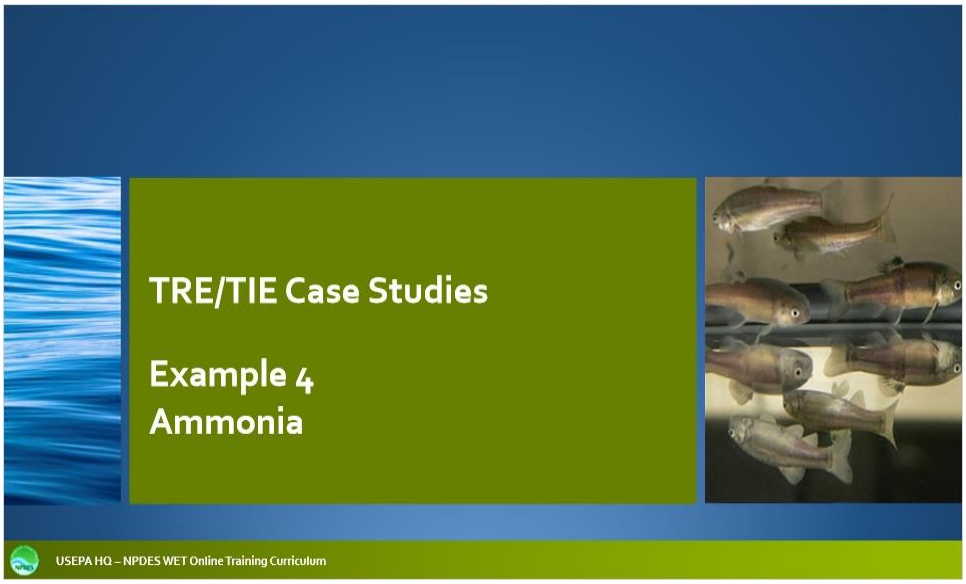
Notes:

TDS is the measure of all dissolved material in water that passes through a 0.2-micron filter. A good indicator that TDS may be the cause of toxicity, but not a certainty, is the conductivity of the sample, with higher TDS that is often associated with higher conductivity. Another indicator of TDS toxicity is the response observed in toxicity tests with *Ceriodaphnia dubia* or *Daphnia pulex* as compared with *Daphnia magna*. *Daphnia magna* are more tolerant to higher TDS than the other two species and, therefore, elicits a less sensitive response. When TIEs indicate that TDS may be the cause of toxicity there may be no toxicity reduction or precipitate formed when adjusting the effluent with either high- or low-pH treatment. Toxicants may or may not be removed with ion exchange resins. Also mock or synthetic effluents that mimic the ion composition of the effluent sample will often produce similar toxicity.



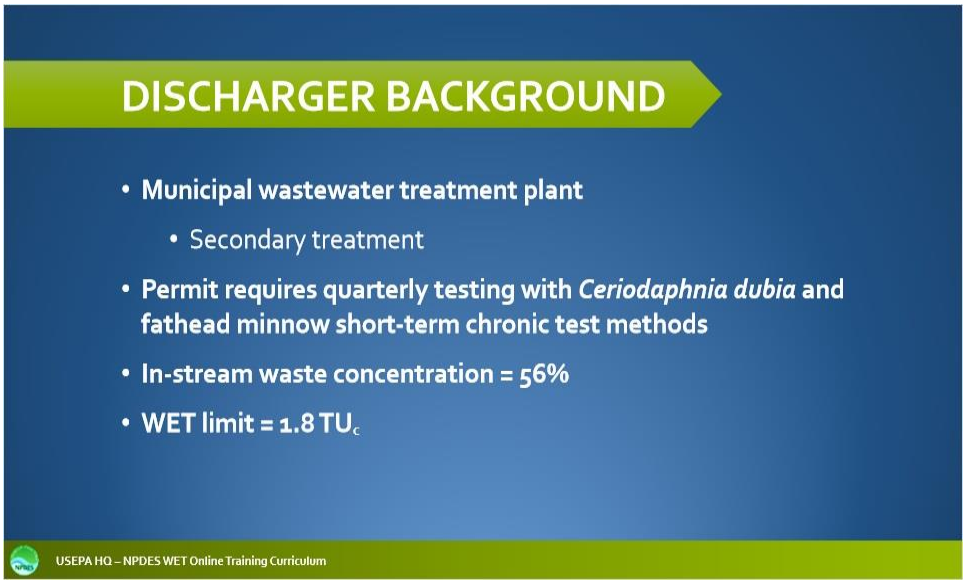
Notes:

The toxicity of this municipal effluent was almost exclusively observed when sample conductivity was greater than 1,000 µS/cm. Conductivity over 1,000 µS/cm is often toxic to *Ceriodaphnia dubia*, but not *Daphnia magna*, which is more tolerant to ionic strength and shows a toxic response at higher conductivity. By conducting chemical analysis, the permittee was able to conclude that chloride was the largest contributor to the overall TDS. The laboratory was able to develop a mock effluent that mimicked the ion concentrations measured in the effluent. Similar toxicity was observed in the mock effluent compared to the actual effluent, thus indicating TDS, specifically ions such as chloride, may be the cause of observed toxicity. In addition, in this TIE, calcium was added to the effluent and the mock effluent and reductions in toxicity were observed in both cases. It is known that where the anion chloride is in solution with the two cations, Ca^{2+} and Na^{2+}, the sample will have reduced toxicity. Thus, by adding calcium, the reduced toxicity observed supported chloride as the leading cause.



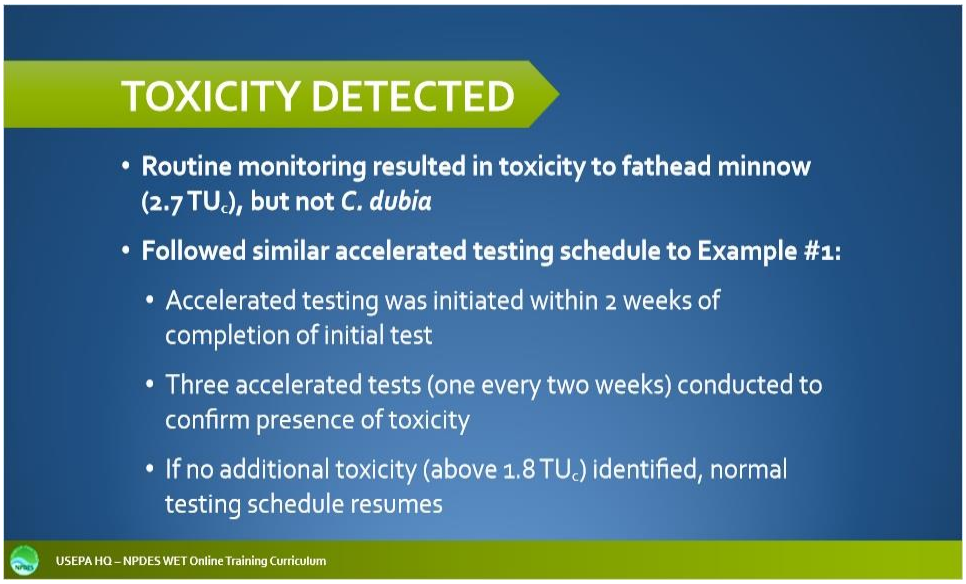
Notes:

In this example, we will look at a municipal effluent that had a toxicity issue involving ammonia.



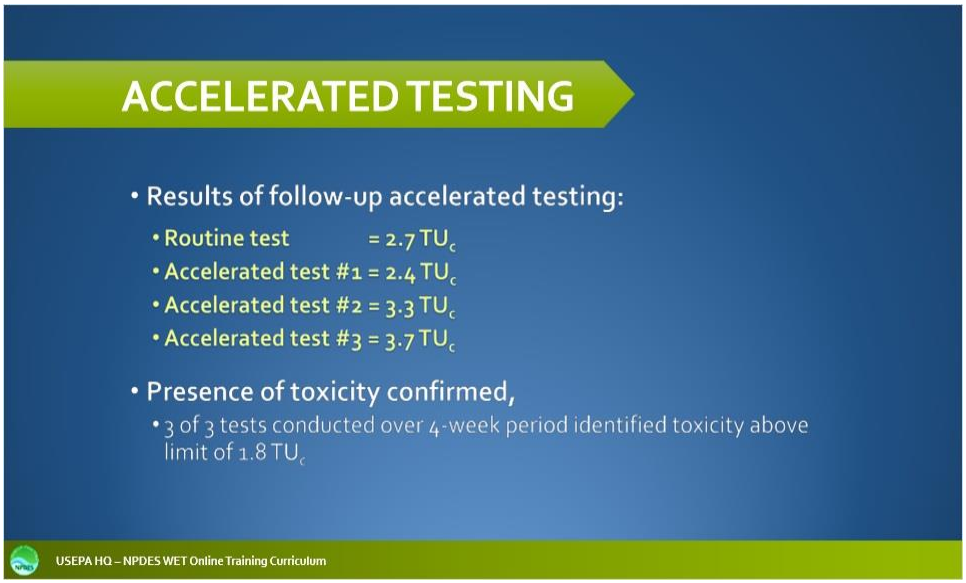
Notes:

In this example the discharger is a municipal wastewater treatment plant with secondary treatment. Under the NPDES permit, the discharger is required to conduct quarterly WET testing using the freshwater water flea (*Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*). The chronic in-stream waste concentration, or critical dilution, is 56% effluent. Therefore, the WET limit is 1.8 TU_c or 100%/56%.



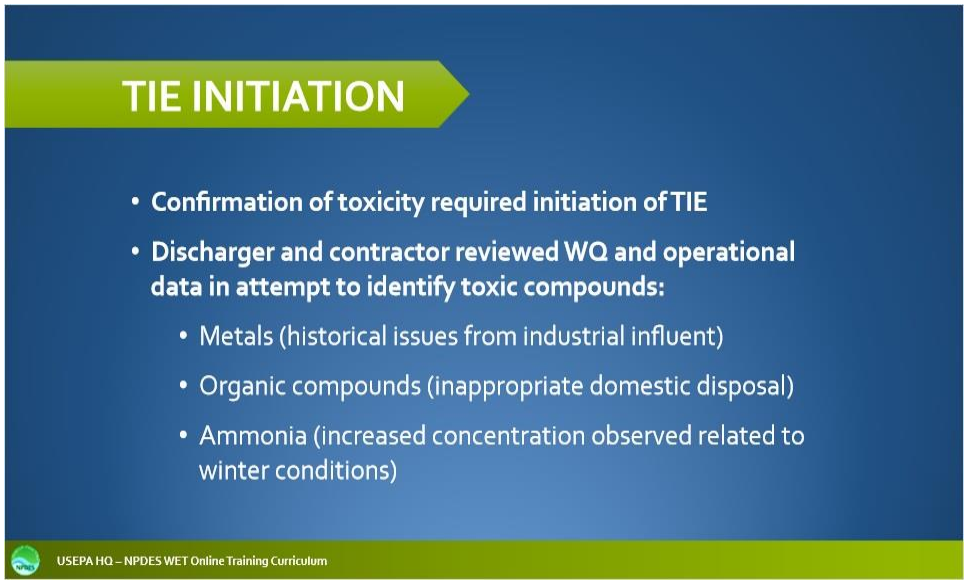
Notes:

Under the dischargers' NPDES permit, routine monitoring is conducted quarterly and during one of those routine monitoring rounds, toxicity was observed to the fathead minnow and not *Ceriodaphnia dubia*. The chronic toxicity unit, TU_c, calculated for the fathead minnow was 2.7, thus equivalent to an IC_{25} of 37% effluent. Based on the accelerated testing clause in the NPDES permit, the discharger was required to begin accelerated testing within two weeks of completing the initial test where toxicity was observed. The accelerated test schedule consisted of three additional tests, one every two weeks to confirm the presence and persistence of toxicity. If no additional toxicity was observed in the accelerated testing, then the discharger would return to the normal quarterly monitoring.



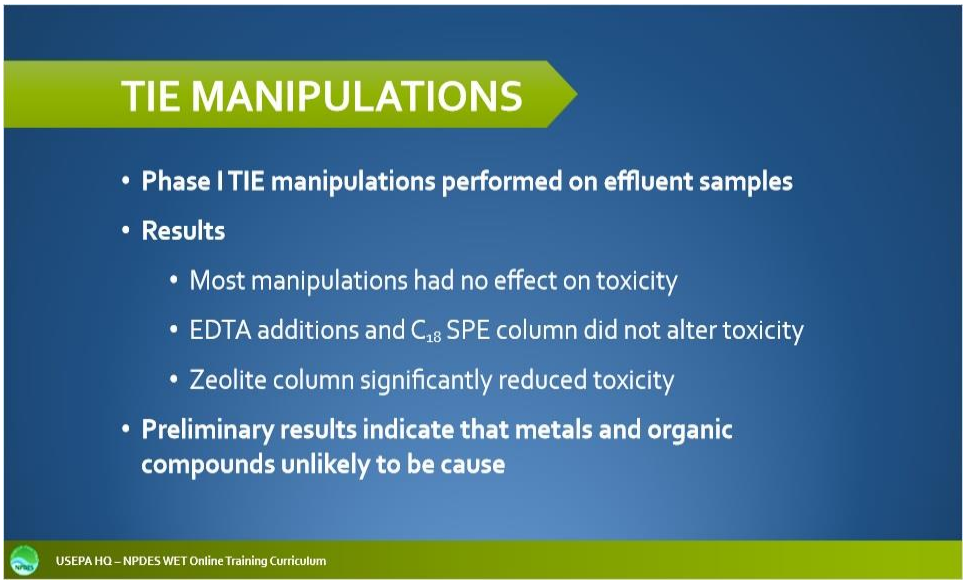
Notes:

During the three accelerated WET tests, toxicity continued to be observed and actually increased in magnitude up to 3.7 TU_c, or an IC_{25} of 27% effluent. The presence and persistence of toxicity in the effluent was observed in all three of the accelerated tests conducted over a four-week period in which toxicity was above the permit limit of 1.8 TU_c.



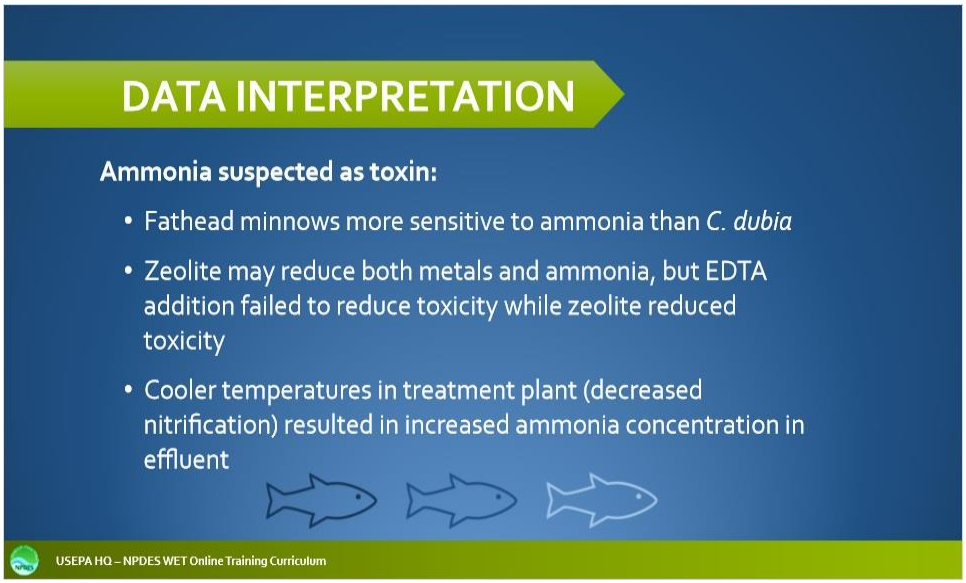
Notes:

After conducting the accelerated monitoring and confirming the presence and persistence of effluent toxicity, the discharger initiated a toxicity reduction evaluation and as part of the TRE initiated a toxicity identification evaluation, TIE. One of the initial steps outlined in the TRE work plan submitted by the discharger to the permitting authority was a review of water quality and operational data over the period of observed toxicity to attempt to identify the toxic compound. The review noted the discharger had a previous history of metals from the influent of an industrial indirect discharger. Additionally, an organic compound from inappropriate household disposal was suspected as well as increased ammonia concentrations during winter conditions.



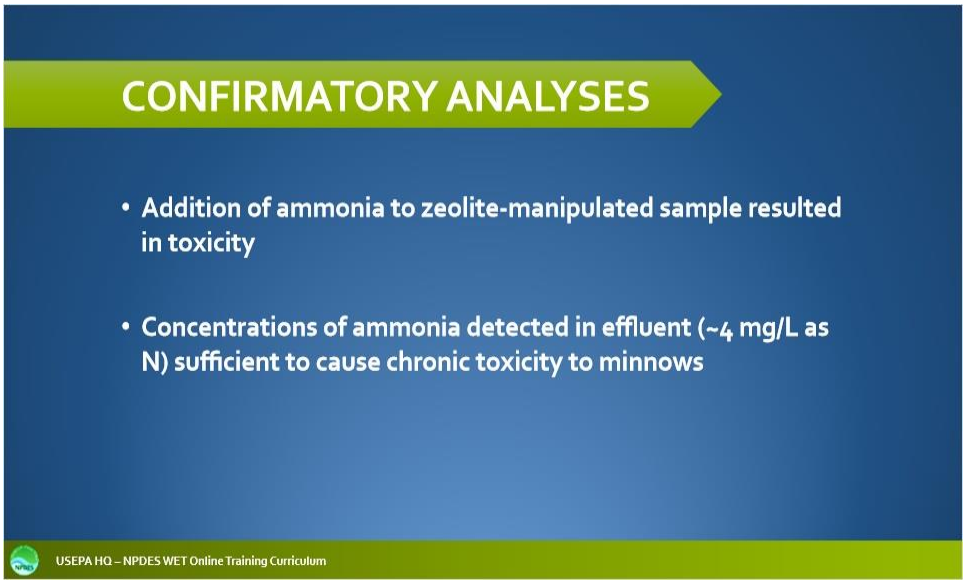
Notes:

The next step, after the initial review of the water quality and operations data, was the initiation of Phase I TIE manipulations including treatments with EDTA, sodium thiosulfate, pH adjustments, filtration, aeration, and C_{18} SPE column. Most Phase I TIE manipulations had little to no effect on toxicity including the EDTA and C_{18} manipulations. An additional manipulation was conducted using zeolite, which removes ammonia as well as some other constituents (e.g., cationic metals), which resulted in a significant reduction in toxicity. Preliminary results indicated that two of the three potential sources, metals and organic compounds, identified in the initial water quality and operations review, were unlikely to be the cause of the observed toxicity.



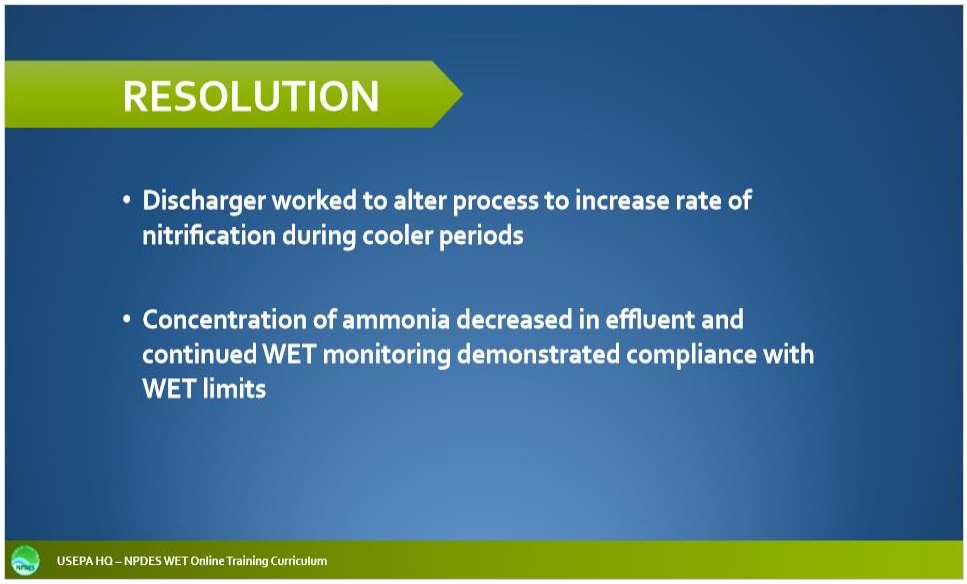
Notes:

Based on several lines of evidence including, fathead minnows being more sensitive than *Ceriodaphnia dubia*; zeolite reducing toxicity but EDTA not reducing toxicity, and overall winter conditions reducing nitrification and increasing the concentration of ammonia in the effluent, ammonia was identified as the potential cause of toxicity.



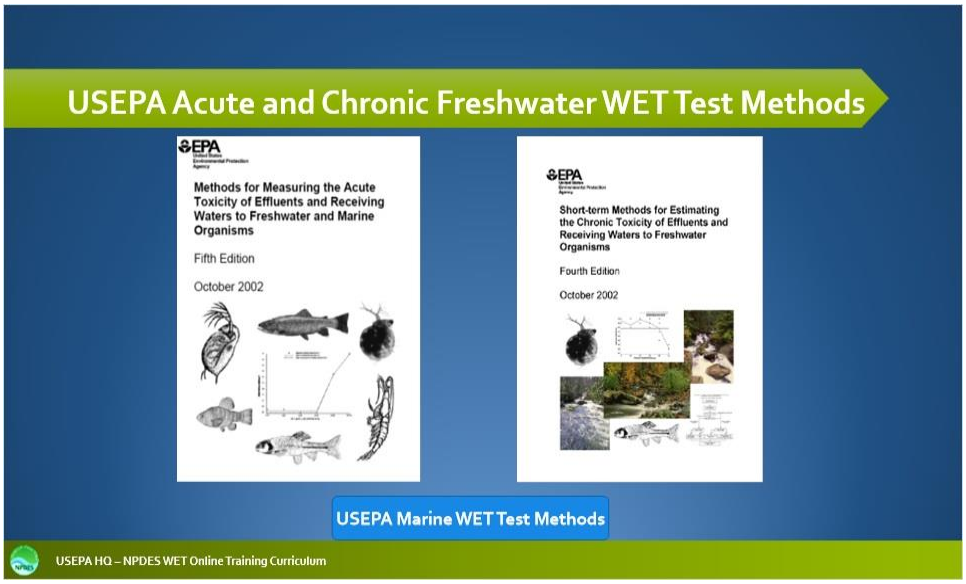
Notes:

As a follow-up to the Phase I manipulations and as part of the confirmatory analyses, ammonia was added back into a zeolite treated effluent sample and similar toxicity was observed as in the original effluent sample. The discharger had been measuring increased ammonia concentration in the discharge during the winter and an effluent concentration of approximately 4 mg/L as nitrogen is sufficient to cause chronic toxicity to minnows.



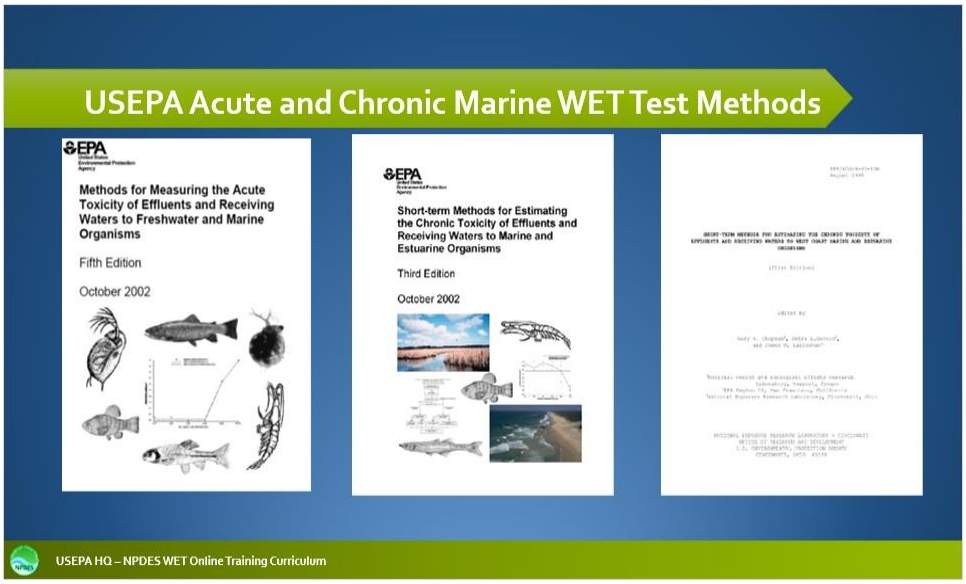
Notes:

Based on the TIE results, the discharger along with the WET laboratory and permitting authority modified the treatment process to ensure increased rates of nitrification even during cooler periods of the year. After this modification, the measured effluent ammonia concentration decreased, and the WET monitoring demonstrated compliance with the WET permit limits. By evaluating the operations data, the discharger was able to assist in identifying the cause of toxicity as well as return to compliance with the permit limits through a slight change in operations.



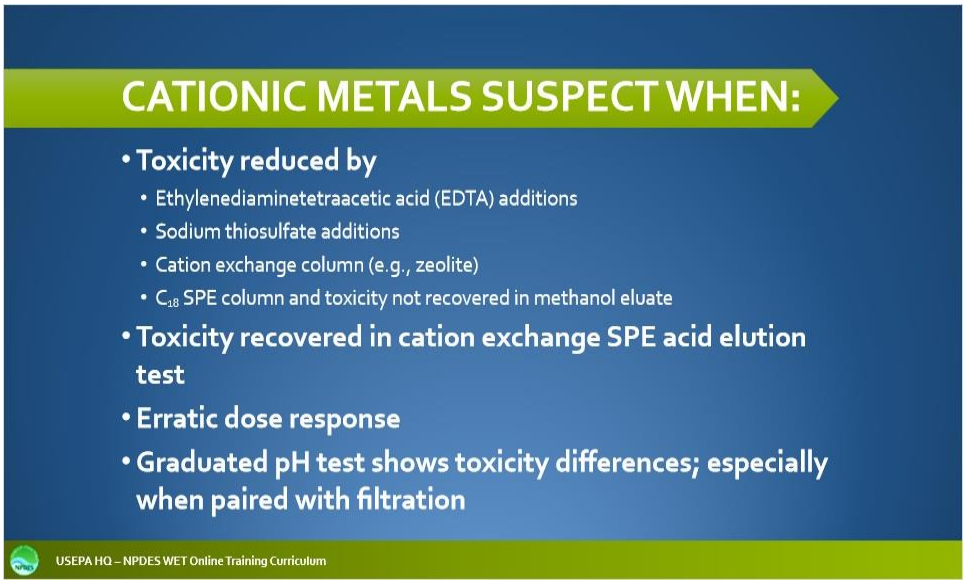
Notes:

The base module presented here examines USEPA's freshwater acute WET test methods entitled "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms", Fifth Edition, EPA-821-R-02-012, hereafter acute toxicity test methods. In addition, this module provides USEPA's short-term chronic freshwater WET test methods entitled "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms", Fourth Edition, EPA-821-R-02-013, hereafter chronic toxicity test methods.



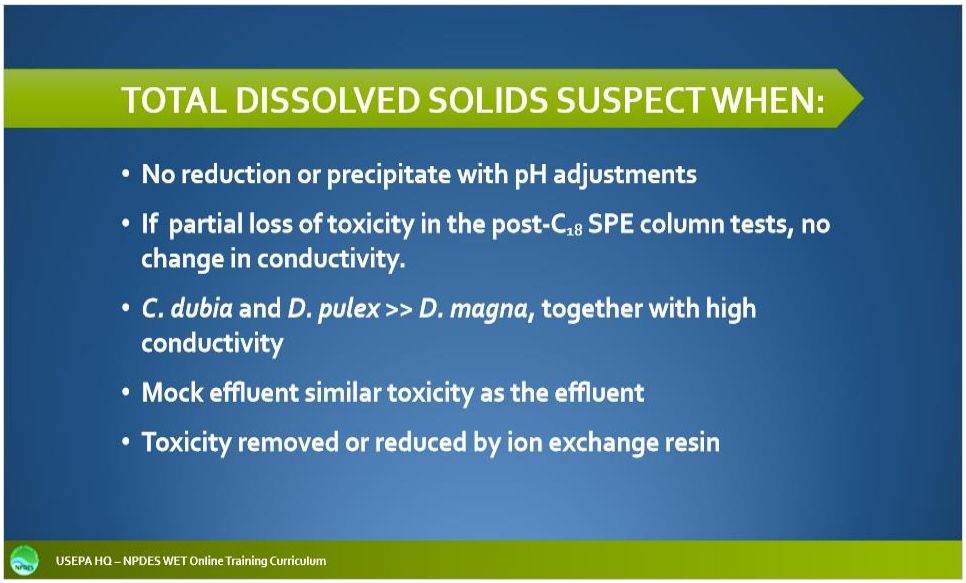
Notes:

This course also provides an opportunity to view USEPA's acute marine WET test methods entitled "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms," Fifth Edition, EPA-821-R-02-012; short-term chronic marine WET test methods used by states on the Atlantic Ocean or Gulf of Mexico entitled "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms," Third Edition, EPA-821-R-02-014, hereafter East Coast test methods; or short-term chronic marine WET test methods used by states on the Pacific Ocean entitled "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms," First Edition, EPA-600-R-95-136, hereafter West Coast test methods.



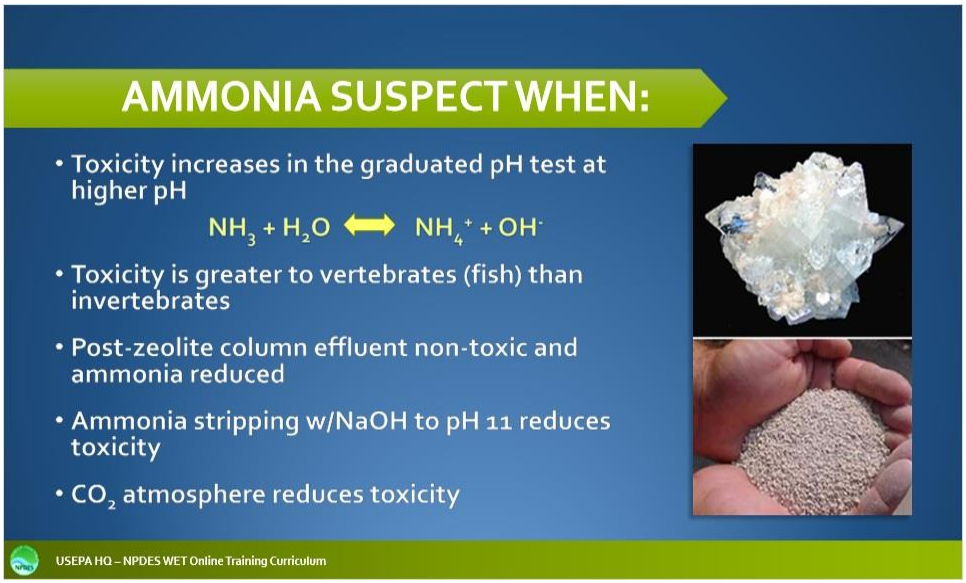
Notes:

The presence of cationic metals, those metals that form positively charged ions, may be suspected in the TIE when certain treatments indicate a reduction in effluent toxicity. The addition of ethylenediaminetetraacetic acid, or EDTA, will generally cause a reduction in the toxicity associated with the sample when cationic metals are the main cause of toxicity. Other TIE treatments that may reduce effluent toxicity when cationic metals are the cause of toxicity include the addition of sodium thiosulfate, the use of cation exchange columns, such as zeolite, and the lack of toxicity recovery in the methanol elution from an C_{18} SPE column but recovered in acid elution from C_{18} SPE column. Many times, cationic metals will cause erratic concentration responses and graduated pH tests may show differences in toxicity, especially when used in conjunction with filtration of the effluent.



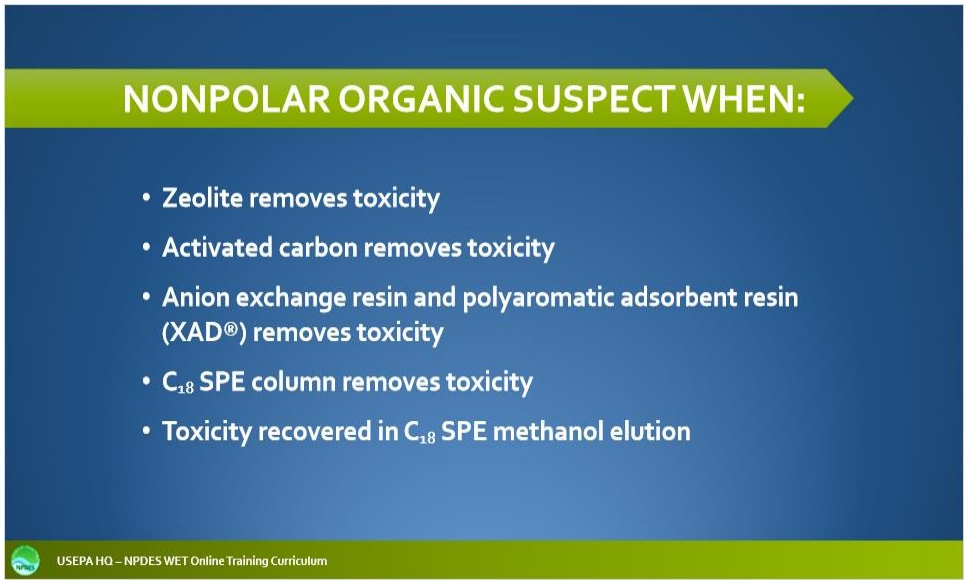
Notes:

Total dissolved solids, or TDS, is the measure of all dissolved material in water that passes through a 0.2-micron filter. TDS can contain a mixture of different ions that don't all behave the same. TDS due to primarily chloride will act differently than TDS due to primarily sulfate or carbonate. In a TIE/TRE it may be important to determine which ions are in the TDS or whether the balance of ions is responsible for the toxicity. A good indicator that TDS could be the cause of toxicity is the conductivity of the sample; higher TDS is often associated with higher ionic strength or conductivity. Another indicator of TDS toxicity is the response observed in invertebrate toxicity tests using the test species *Ceriodaphnia dubia* or *Daphnia pulex* as compared with *Daphnia magna*. *Daphnia magna* are more tolerant of higher TDS than the other two test species, and therefore will exhibit less toxicity. Where TIEs indicate that TDS may be the cause of toxicity, there may be no toxicity reduction or precipitate formed when adjusting the sample using either high or low pH. When TDS is the suspected toxicant, toxicity may or may not be removed with ion exchange resins and mock or synthetic effluents that mimic the ion composition of the sample will produce similar toxicity results when used.



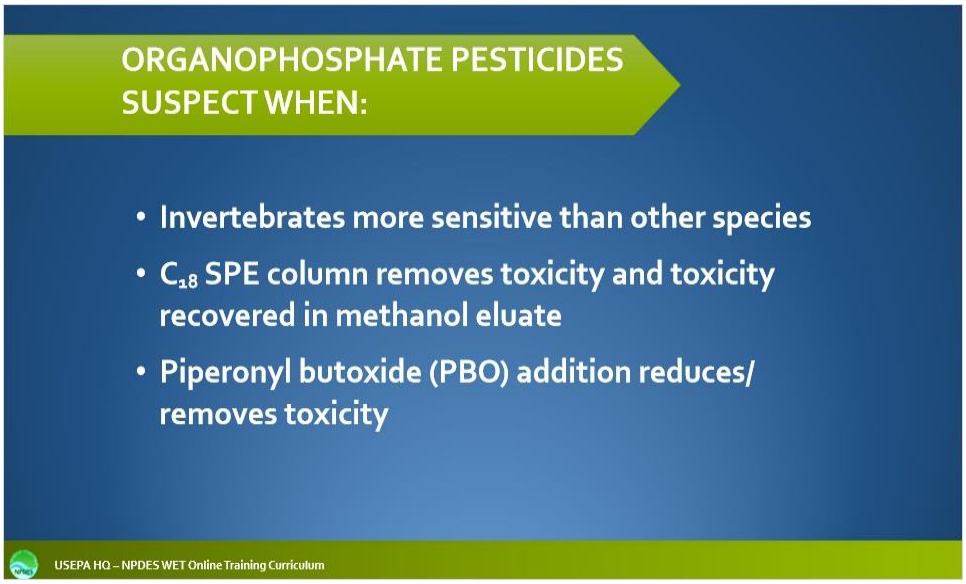
Notes:

When vertebrates (i.e., fish) are found to be more sensitive in toxicity testing than invertebrates to a sample, ammonia is often one of the primary candidate causes of toxicity. Ammonia tends to be more toxic to vertebrates and becomes more toxic as pH increases in the sample. Unionized ammonia is more toxic to aquatic life than the ammonium ion. As pH increases, ammonium ion is converted to unionized ammonia. The higher the pH, the higher the percentage of unionized ammonia. A one-unit difference in pH corresponds to an approximately 10-fold increase in the percentage of unionized ammonia present. TIE manipulations that indicate ammonia as the cause of toxicity include a reduction of toxicity by treating the sample with zeolite. Zeolite is a natural material that binds ammonia. Ammonia stripping with sodium hydroxide at a pH of 11 will typically result in a reduction in toxicity if ammonia is the cause. Use of a carbon dioxide atmosphere in toxicity testing will also reduce toxicity due to ammonia because carbon dioxide in the air above the sample helps prevent any increase in sample pH during the test. Thus, stabilizing the pH of the sample decreases the concentration of unionized ammonia in the sample and therefore decreases the toxicity.



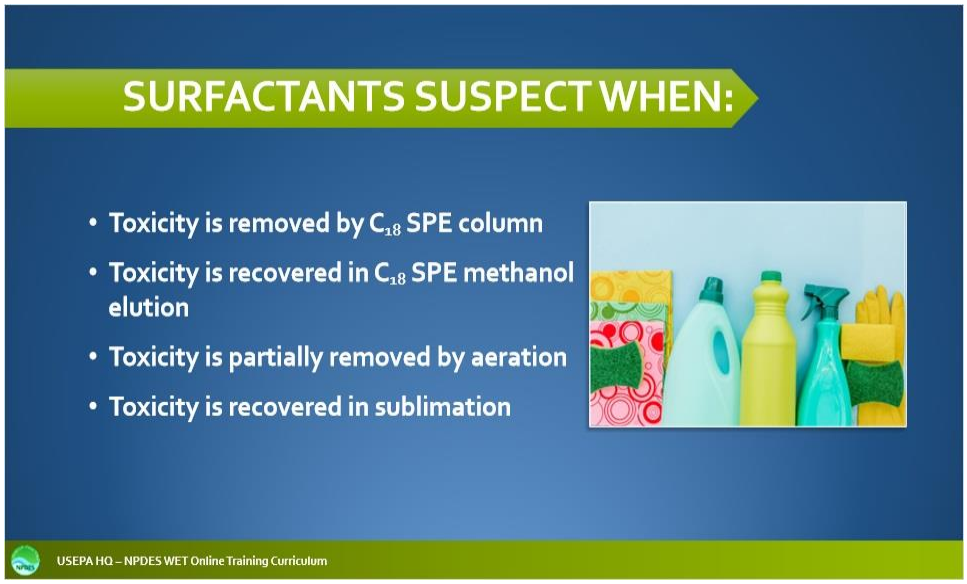
Notes:

In some discharges, nonpolar organics, for example hexane, benzene, or gasoline range organics, may be the cause of toxicity. In conducting a TIE, often multiple treatments will reduce sample toxicity. When nonpolar organics are the cause of toxicity, generally one will see reductions in toxicity when the sample is treated with zeolite, activated carbon, an anion exchange resin, an XAD resin, or a C_{18} SPE column. Additionally, if the C_{18} SPE column removes toxicity of the sample, this can be confirmed if toxicity is again observed after methanol elution of the C_{18} SPE column (where the nonpolar organic chemical that is trapped in the SPE column is released back into what was a non-toxic sample). Further testing would be needed to determine whether a nonpolar organic is the primary source of toxicity and if so, which chemical(s).



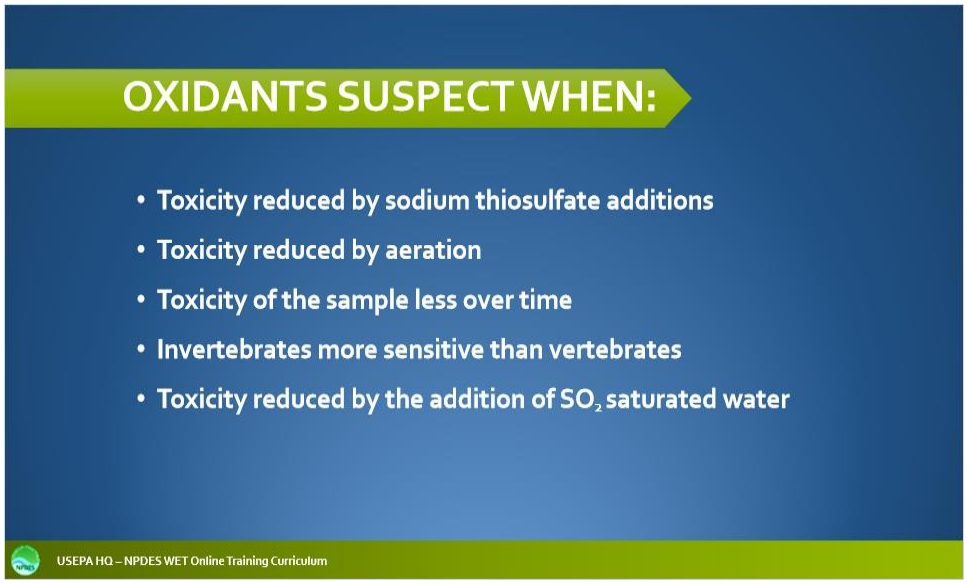
Notes:

When organophosphate pesticides such as diazinon or chlorpyrifos are the suspected toxicant, the invertebrates, particularly crustaceans, such as *Ceriodaphnia dubia*, *Daphnia pulex*, or *Daphnia magna*, will generally be more sensitive than vertebrates. Organophosphate toxicity will generally be reduced when using a C_{18} SPE column and one can remove the toxicants by eluting the SPE column with methanol. There are other more advanced TIE manipulations that will help identify organophosphates as the toxicant, including treatment of the effluent with piperonyl butoxide, or PBO. PBO will tend to reduce or remove the toxicity associated with organophosphates, however, it enhances or increases the toxicity associated with other pesticides such as carbamates, pyrethrins, pyrethroids, and rotenone.



Notes:

Surfactants are compounds that are typically found in cleaning products because of their ability to lower the surface tension of water. Surfactants include soaps and detergents, but also lubricants, inks, anti-fogging liquids, herbicides, adhesives, emulsifiers, and fabric softeners. When conducting a TIE, surfactants may be the suspected cause of toxicity when toxicity is resolved by passing the sample through a C_{18} SPE column, recovered using a methanol elution. Surfactant toxicity may also be reduced by aeration and be recovered via sublimation of the sample. Sublimation refers to the deposition of the recovered material on the sides of an aeration chamber. Toxicants may be recovered using a dilution water or methanol rinse of the aeration chamber.



Notes:

Oxidants are materials that oxidize or remove electrons from other reactants during a redox reaction. Oxidants have been associated with causing toxicity and can be identified in the TIE process. Examples of oxidants include acids (i.e., nitric and sulfuric acid), hydrogen peroxide, sodium perborate, potassium nitrate, and halogens (e.g., chlorine and fluorine). In the TIE process, oxidants may be suspected when the toxicity of the sample is reduced or removed by treatment with sodium thiosulfate, aeration, or if toxicity degrades over time after storage or exposure to air. Typically, invertebrates are more sensitive to oxidants and the toxicity associated with oxidants may be reduced by the addition of sulfur dioxide (SO_2) saturated water. Sulfur dioxide is a dechlorinating agent; thus, if an effluent treated with SO_2 indicates less toxicity, then the toxicity may be due to residual chlorine in the sample.