Exhibit 2

# ALYESKA PIPELINE SERVICE COMPANY STUDIES RELATED TO THE BALLAST WATER TREATMENT FACILITY VALDEZ, ALASKA 

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## Exhibit 3

# Technical Support Document For Water Quality-based Toxics Control 

Table 1-5. Interlaboratory Precision of Nonmetal Inorganic Analyses Over the Measurement Range [15]

| No. <br> Lab | Parameter | $C V(\%)$ Range |
| :---: | :--- | :---: |
| 17 | Alkalinity | $4.9-14$ |
| $>20$ | Residual chlorine | $13-25$ |
| 16 | Ammonia nitrogen | $15-58$ |
| 6 | Kjeldahl nitrogen, total | $38-41$ |
| 15 | $N 0_{3}$ nitrogen | $17-61$ |
| 6 | Total $P$ | $25-40$ |
| 58 | BOD | $15-33$ |
| 58 | COD | $6.9-34$ |
| 21 | TOC | $4.6-70$ |

associated with organic chemical analyses. The CVs range from 12 percent to 91 percent. Table $1-5$ demonstrates the interlaboratory precision of nonmetal inorganic analyses at the lower end of the measurement range. The CVs for this type of analyses range from 4.6 percent to 61 percent [15]. The data in Tables $1-3$ to $1-5$ reflect testing in reagent grade water. Actual CVs from testing effluents can be higher due to matrix effects. However, in 40 CFR Part 136 analytical methods, matrix effects are acknowledged.

### 1.3 WHOLE EFFLUENT APPROACH FOR AQUATIC LIFE PROTECTION

The whole effluent approach to toxics control for the protection of aquatic life involves the use of acute and chronic toxicity tests to measure the toxicity of wastewaters. Whole effluent toxicity is a useful parameter for assessing and protecting against impacts upon water quality and designated uses caused by the aggregate toxic effect of the discharge of pollutants [16]. Whole effluent toxicity tests employ the use of standardized, surrogate freshwater or marine (depending upon the mixture of effluent and receiving water) plants, invertebrates, and vertebrates. EPA has published extensive written protocols listing numerous marine and freshwater species for toxicity testing $[17,18,19]$.

An acute toxicity test is defined as a test of 96 -hours or less in duration in which lethality is the measured endpoint. A chronic toxicity test is defined as a long-term test in which sublethal effects, such as fertilization, growth, and reproduction, are usually measured, in addition to lethality. Traditionally, chronic tests are full life-cycle tests or a shortened test of about 30 days known as an early life stage test. However, the duration of most of the EPA chronic toxicity tests have been shortened to 7 days by focusing on the most sensitive life-cycle stages. For this reason the EPA chronic tests are called short-term chronic tests. Box 1-2 summarizes the short-term chronic tests currently recommended by EPA. The acute and short-term chronic methods recommended by EPA are presented in three methods manuals $[17,18,19]$.

In a laboratory acute toxicity test, an effluent sample is collected, diluted, and placed in test chambers with the chosen test species. After 24, 48, 72, and 96 hours, the number of live organisms remaining in each test concentration and in a control is recorded. In a laboratory chronic toxicity test, an effluent sample is collected, diluted, and placed in test chambers. An example of a dilution series used in chronic or acute tests is $100,50,25,12.5$, and 6.25 percent, and a control. Test organisms are placed in these test chambers for specified periods of time. At various times during the exposure period, the organisms in each chamber are observed. In the short-term chronic tests, at test termination, the lowest effluent concentration that causes a significant adverse impact on the most sensitive endpoint for that test is calculated (this endpoint can be mortality, reduced fertilization, lower fecundity, reduced growth, etc.). In the acute tests, at test termination, the number of dead organisms are recorded and an $L C_{50}$ is calculated.

Dilution water is an important part of toxicity testing. Dilution water may either be standard laboratory water and/or the receiving water. Sometimes the receiving water is used to dilute the effluent because it more closely simulates effluent/receiving water interactions. This may be especially important in the case of saline receiving waters. The salinity of the receiving water should be matched as closely as possible to the salinity in the test chambers (within the salinity range constraints of a particular method) for the purposes of conducting the tests.

Quality control and quality assurance are an integral part of whole effluent toxicity testing. Use of a standard control water and a reference toxicant test are both recommended to ensure quality assurance in chronic testing. It is important to understand that each of the chronic tests has minimum criteria of acceptability for each endpoint that is measured in the controls (i.e., 80 percent survival and minimum criteria for growth, reproduction, and fertilization). The acute tests also have criteria of acceptability measured in the controls.

Acute toxicity endpoints (ATEs) commonly include lethal concentrations (LCs) and are described in terms of effluent concentrations. The LC is the concentration of toxicant at which a certain percentage of the test organisms die, e.g., the $\mathrm{LC}_{10}$ or $\mathrm{LC}_{50}$. An exposure duration also is included in the endpoint such as 24,48 , 72, or 96 hours (e.g., 96 -hour LC $C_{50}$ ).

Commonly used chronic toxicity endpoints (CTES) include the no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), and the effect concentration (EC). The NOEC is the highest concentration of toxicant, in terms of percent effluent, to which the test organisms are exposed that causes no observable adverse effect. The effects measured may include decreases in reproduction and growth, or lethality. The LOEC is the lowest concentration of toxicant to which the test organisms are exposed that causes an observed effect. Again, the same effects are usually observed. The EC is the toxicant concentration that would cause an adverse effect upon a certain percentage of the test organisms, (e.g., $\mathrm{EC}_{10}$ or $\mathrm{EC}_{50}$ ).

In chronic toxicity tests, the exposure duration in the EPA testing protocols is almost always assumed to be the 7 -day short-term period unless otherwise specified in the protocol. For example, the Ceriodaphnia test must be continued until at least 60 percent

| Box 1-2. Short-term Chranic Toxicity Methods |  |  |
| :---: | :---: | :---: |
| Species/Common Name | Test Duration | Test Endpoints |
| Freshwater Species |  |  |
| Ceriodaphnia dubia | Approximately 7 days | Survival, reproduction |
| Cladoceran | (until 60 percent of control have 3 broods) |  |
| Pimephales promelas | 7 days | Larval growth, survival |
| Fathead minnow |  |  |
| Pimephales promelas | 7-9 days | Embryo-larval survival, |
| Fathead minnow |  | percent hatch, percent abnormality |
| Selenastrum capricornutum | 96 hours | Growth |
| Freshwater algae |  |  |
| Marine/Estuarine Species |  |  |
| Arbacia punctulata | 1.5 hours | Fertilization |
| Sea urchin |  |  |
| Champia parvula | 7.9 days | Cystocarp production |
| Red macroalgae |  | (fertilization) |
| Mysidopsis bahia | 7 days | Growth, survival, fecundity |
| Mysid |  |  |
| Cyprinodon variegatus | 7 days | Larval growth, survival |
| Sheepshead minnow |  |  |
| Cyprinodon variegatus | $7-9$ days | Embryo-larval survival, |
| Sheepshead minnow |  | percent hatch, |
|  |  | percent abnormality |
| Menidia beryllina | 7 days | Larval growth, survival |
| Inland silverside |  |  |

of the females produce three broods. This may require more or less than 7 days to occur.

It is useful to note that LCs and ECs are point estimates statistically derived from a mathematical model that assumes a continuous dose-response relationship. NOECs and LOECs, statistically determined using hypothesis testing, are not point estimates [18]. In order to overcome the difficulty in statistically deriving the NOEC using hypothesis testing, a new statistical procedure has been developed. This procedure, referred to as the inhibition concentration (IC), is a point estimate interpolated from the actual
effluent concentrations at which measured effects occurred during a chronic test. The IC is an estimate of the toxicant concentration that would cause a given percent reduction in a biological measurement of the test organisms, including reproduction, growth, fertilization, or mortality. For example, an $\mathrm{C}_{25}$ for reproduction would represent the effluent concentration at which a 25 -percent reduction in reproduction occurred.

Since the IC is a point estimate, a CV can be calculated. A CV cannot be calculated if hypothesis testing is used because results are only available for the effluent concentrations used. For this
reason, estimates of test precision cannot be calculated for NOECs derived by hypothesis testing.

The IC also is not dependent upon the selection of the effluent concentrations. In contrast, NOECs calculated by hypothesis testing are dependent upon the concentrations initially selected. For example, if a chronic test is conducted using 100,50,25, 12.5 , and 6.25 percent effluent concentrations, and the LOEC exhibited by the data is at 25 percent effluent, the NOEC calculated by hypothesis testing is estimated to be the next lowest dilution, or 12.5 percent. However, the true NOEC value may lie somewhere between 25 percent and 12.5 percent effluent.

Comparisons of both types of data indicate that an NOEC derived using the $\mathrm{IC}_{25}$ is approximately the analogue of an NOEC derived using hypothesis testing (see Figure 1-1). For the above reasons, if possible, the $\mathrm{IC}_{25}$ is the preferred statistical method for determining the NOEC.

Another important issue in conducting both acute and short-term chronic toxicity tests is the dilution series. The EPA methods manuals recommend six dilutions, including the control. The only exception to this is a toxicity test conducted on ambient receiving waters. Then, each ambient receiving water is compared statistically to the control without dilutions. It is not accurate to assume that two dilutions (the receiving water concentration [RWC] and control) are all that are ultimately necessary for determining compliance with a toxicity limit. If the toxicity tests are conducted with only the control and one effluent concentration (i.e., the RWC), the error and variability associated with this type of statistical analysis is large [20].

For the above reasons, EPA recommends the use of five effluent concentrations and a control to determine the magnitude of toxicity. When conducting compliance monitoring, an option is to choose the five concentrations that bracket the RWC (two concentrations above and two below). This would result in the determination of compliance status as well as a statistically valid estimation of the NOEC. The information provided from the full dilution series would indicate how close the test endpoints are to the permit limit and how close to violating the limit the discharger is, and, if measured over time, the variability of the effluent.

### 1.3.1 Tourd Units

Since toxicity involves an inverse relationship to EC (the lower the EC , the higher the toxicity of the effluent), it is more understandable to translate concentration-based toxicity measurements into toxic units (TUs). In this way, the potential confusion involving the inverse relationship is overcome and the permit limit derivation process is better served. The number of toxic units in an effluent is defined as 100 divided by the EC measured:

$$
\begin{aligned}
& T U_{a}=100 / L C_{S 0} \\
& T U_{C}=100 / \mathrm{NOEC}
\end{aligned}
$$

For example, an effluent with an acute toxicity of an $\mathrm{LC}_{50}$ in 5 percent effluent is an effluent containing $20 \mathrm{TU}_{\mathrm{a}} \mathrm{s}$.

A very important aspect of toxic units is that two different types are used depending on whether acute or chronic aquatic toxicity is measured. The proper expressions for toxic units are $\mathrm{TU}_{\mathrm{a}}$ and


Figure 1-1. This figure represents the percentage of the time the mean NOEC was approximately equivalent to an $I C_{10}, I C_{15}, I C_{20}$, $I C_{25}, I C_{30}$, and $I C_{50}$ for all 23 effluent and reference toxicant data sets analyzed. The data sets included short-term chronic toxicity test for Ceriodaphnia dubia, Pimephales promelas (fathead minnows), Arbacia punctulata (sea urchin), Cyprinodon variegatus (sheepshead minnows), and Champia parvula (red algae) [21].
$T U_{c} . \mathrm{TU}_{a}$ is the measurement of acute toxicity units and $\mathrm{TU}_{c}$ is a measurement of chronic toxicity units. (See the glossary for a definition of these terms.) They are not the same measurement and should not be used interchangeably. Acute and chronic TUs make it easy to quantify the toxicity of an effluent and to specify water quality criteria based upon toxicity. For example, an effluent sample that contains $20 \mathrm{TU}_{c}$ s is twice as toxic as an effluent that contains $10 \mathrm{TU}_{\mathrm{c}} \mathrm{s}$.

### 1.3.2 Correlation of Whole Effluent Toxicity Measuroments to Actual Receiving Wator mpact

EPA conducted the Complex Effluent Toxicity Testing Program (CETTP) that examined sites in both freshwater and saltwater systems to investigate whether or not an evaluation of effluent toxicity, when adequately related to receiving water conditions (i.e., temperature, pH , salinity), can give a valid assessment of receiving system impacts on waters that support aquatic biota [22-25]. Summaries of these site studies are provided in Box 1-3 (freshwater) and Box 1-4 (saltwater). In addition, three other studies, presented in Box 1-3, were conducted to address this issue: a comparative investigation conducted by the University of Kentucky [26], a second study on the Trinity River in Texas conducted by the University of North Texas [27], and a third study conducted by the North Carolina Division of Environmental Management [28].

It is important to note that in these studies, different objectives were addressed. The CETTP freshwater studies attempted to correlate receiving water chronic toxicity measured by EPA toxicity tests to instream observed impacts (Figure 1-2). The CETTP saltwater studies compared effluent toxicity to ambient receiving water toxicity using dye studies to measure receiving water concentrations of effluent. The North Carolina study compared
effluent toxicity to receiving water impact using Ceriodaphnia chronic toxicity tests and receiving stream benthic macroinvertebrates (Figure 1-3). The Kentucky study examined the relationship between effluent toxicity tests and instream ecological parameters. The Trinity River study attempted to spatially compare the biological, physical, and chemical water quality and sediment quality of Trinity River reaches above and below the Dallas/Fort Worth area (Figure 1-4).

Together, these studies comprise a large data base specifically collected to determine the validity of toxicity tests to predict receiving water community impact. In order to address the correlation of effluent and ambient toxicity tests to receiving water impacts, EPA evaluated the results of the studies discussed above [29]. The results, when linked together, clearly show that if toxicity is present after considering dilution, impact will also be present.

Parkhurst et al., were requested by representatives of industrial and municipal discharges to critique the CETTP studies [30]. One major criticism was that the EPA study sites were not selected randomly and therefore the results of the studies cannot be extended to all waters. EPA agrees that the CETTP sites were not selected to represent a statistically valid sampling of all types of waterbodies in the United States. A representative sampling of receiving water would require assessment of more sites than EPA could study in a comprehensive manner. Such a sampling was beyond the capability of EPA's resources. However, the CETTP and corresponding studies such as the Trinity River study [27] did show unequivocally that a strong correlation exists between toxicity and a biological impact.

EPA believes that it is reasonable to assume in the absence of data showing otherwise that this relationship is basically independent

## Box 1-3. Correlation of Toxicity Measurements to Receiving Water Impact (Freshwater)

EPA conducted eight freshwater site studies in which ambient toxicity was compared to the receiving water biological impact. These site studies were a part of the Complex Effluent Toxicity Testing Program (CETTP). Testing was done onsite concurrent with the field surveys. Sites exhibiting biological impacts in Oklahoma, Alabama, Maryland, West Virginia, Ohio, and Connecticut were included. Organisms were exposed to samples of water from various stations and tested for toxicity. Biological surveys (quantitative field sampling of fish, invertebrate, zooplankton, and periphyton communities in the receiving water areas upstream and downstream of the discharge points) were made at these stations at the same time the toxicity was tested to see how well the measured toxicity correlated to the health of the community. These studies have been reviewed and published in the EPA publication series [23, 31-38].

Figure 1-2 illustrates the data from the CETTP studies. A robust canonical correlation analysis was performed to determine whether or not statistically significant relationships existed between the ambient toxicity tests and instream biological response variables and to identify which variables played an important role in that relationship [29]. Influential variables were then used to classify stations as either impacted or not. Ceriodaphnia dubia productivity and/or Pimephales promelas weight were used as the basis for predicting impact. Fish richness was used to classify streams as impact observed or impact not observed.


Figure 3-3. National Distribution of NPDES Dilution Conditions at 7Q10 and at Annual Mean Flow

The changes to the EPA's data generation recommendations eliminate the application of multiple sets of safety margins that was proposed in the 1985 version of this document. Rather, general observations on effluent toxicity described above now allow regulatory authorities to tighten the bounds of the initial dilution categorization, eliminate the species sensitivity uncertainty factor and target $L C_{50}$ of 1 percent and NOECs of 0.1 percent as the most extreme toxicity measurements that can normally be expected for the vast majority of effluents discharged by NPDES permittees for acute and chronic toxicity, respectively. The observation of toxicity was based on multiple dilution tests. The same observation may not hold for toxicity measured with single dilution tests (pass/fail). As reflected in Chapter 1, single
dilution toxicity tests are much more variable than multiple dilution tests. Therefore, the use of single concentration toxicity tests is strongly discouraged for this data generation process.

Since the new data generation requirements are much less expensive than the previous requirements, tiered testing (less expensive, single-concentration, initial screening followed by increasingly expensive definitive data generation, using multiconcentration tests, as described in the September 1985 version of the technical support document) is unnecessary. However, elimination of the requirement to conduct toxicity testing on the basis of projections using dilution alone is not recommended. Although EPA's data review suggests that an $\mathrm{LC}_{50}$ of 1 percent and an NOEC of 0.1 percent are the lower bounds on effluent toxicity, there may be other effluents that are presently unmeasured that are more toxic. Testing data are always desirable for fully characterizing discharges of concern.

## Steps in Whole Effluent Characterization Process

The following is a detailed description of the major steps presented in Figure 3-2 and the rationale behind each.

## Step 1: Dilution Determination

The initial step is to determine the dilution of the effluent at the edge of the mixing zone, assuming the State allows mixing zones. Figure 3-4 shows a schematic representation of typical mixing zone requirements for both acute and chronic toxicity. Calculating the dilution at the edges of mixing zones for site-specific situations can be complicated. Modeling can be employed using either steady-state or dynamic approaches to calculate the dilution (see Chapter 4). However, for complex situations, such as marine and estuarine waters or lakes, dye studies (or other techniques used to assess mixing zones) may still be required.

Some State water quality standards do not allow the use of mixing in the control of acute toxicity. For these States, acute toxicity is often limited at the end of the pipe. Permit limits derived to enforce such requirements would be considered "water quality-based" because they would be based upon an ambient criterion (as opposed to an arbitrary test endpoint). Regardless, both chronic and acute toxicity must be assessed in these situations.

## Step 2: Toxicity Testing Procedures

Where toxicity tests are required in order to make decisions regarding appropriate next steps in a screening protocol, EPA recommends as a minimum that three species (for example, a vertebrate, an invertebrate, and a plant) be tested quarterly for a minimum of 1 year. As discussed in Chapter 1 , the use of three species is strongly recommended. Experience indicates that marine algae can be a highly sensitive test species for some effluents. Using a surrogate species of the plant kingdom adds another trophic level to the testing regimen. For both freshwater and marine situations, the use of three species is more protective than two species since a wider range of species sensitivity can be measured. EPA is continuing to develop toxicity test methods using additional organisms including plants. In addition, EPA has revised the test for Selenastnum, which has improved the test precision.

No initial mixing aliowed:

where

$$
\begin{aligned}
& \mathrm{CMC}=0.3 \mathrm{TU}_{\mathrm{a}} \\
& \mathrm{CCC}=1.0 \mathrm{TU}_{\mathrm{C}}
\end{aligned}
$$

Figure 3-4. Schematic Representation of Mixing Zone Areas Where the CMC and CCC Apply

EPA recommends against selecting a "most sensitive" species for toxicity testing. For one organism to consistently be the most sensitive in a battery of toxicity tests, two conditions must occur: (1) the toxicants causing toxicity must remain the same, and (2) the ratios of the toxicants in the effluent (if more than one) must remain the same. Based on EPA's experience at the Duluth research laboratory, neither of these conditions is likely to occur. For example, the causes of effluent toxicity in POTWs can vary on a seasonal basis. Toxicity in the summer can be caused by pesticides to which invertebrates are most sensitive. However, the winter toxicity could be caused by ammonia to which fathead minnows will respond most sensitively. The most sensitive species for an effluent actually may not exist and at best is difficult to identify.

Conducting toxicity tests using three species quarterly for 1 year is recommended to adequately assess the variability of toxicity observed in effluents. Below this minimum, the chances of missing toxic events increase. The toxicity test result for the most sensitive of the tested species is considered to be the measured toxicity for a particular effluent sample.

The data generation recommendations in Figure 3-2 represent minimum testing requirements. Since uncertainty regarding whether or not an effluent causes toxic impact is reduced with more data, EPA recommends that this test frequency be increased where necessary to adequately assess effluent vari-
ability. If less frequent testing is required in the permit, it is preferable to use three species tested less frequently than to test the effluent more frequently with only a single species whose sensitivity to the effluent is not well characterized.

EPA recommends that a discharger conduct acute toxicity testing if the dilution of the effluent is greater than 1000:1 at the edge of the mixing zone [3]. Such a discharger would be considered a low priority for chronic toxicity testing. The rationale for this is that the effluent concentration would be below 0.1 percent at the edge of the mixing zone and thus incapable of causing an excursion above the CCC. A worst case NOEC of 0.1 percent translates into $1,000 \mathrm{TU}_{\mathrm{C}}$, which would result in a concentration of less than $1.0 \mathrm{TU}_{\mathrm{C}}$ at the edge of the mixing zone for this dilution category. The test results would be compared to the CMC after consideration of any allowable mixing.

EPA recommends that a discharger conduct either acute or chronic toxicity testing if the dilution of the effluent falls between 100:1 and 1,000:1 at the edge of the mixing zone. Effluents have been shown to be both acutely and chronically toxic within this range of receiving water dilution. Under worst-case scenarios, $\mathrm{LC}_{50^{5}}$ of 1.0 percent and ACRs of 10 will result in excursions above both the CCC and CMC at the edge of the regulatory mixing zone.

Although either acute or chronic testing can be required within this dilution range, acute testing would be more appropriate at the higher end of this dilution range ( $1,000: 1$ or 0.1 percent). At the lower end of this dilution range (100:1 or 1.0 percent), chronic tests may be more appropriate. Where other factors are equal, chronic testing may be preferable since the interim results in a chronic test gives data on acute toxicity as well. The acute endpoint data can then be used to compare directly to the CMC without the need for an ACR.

Whichever type of toxicity test (either acute or chronic) is specified, the results from that test should be compared to the criterion associated with that type of test. For example, a chronic test would be compared to the CCC. Comparisons to the other criteria can be made by using the ACR or additional data generated to convert a chronic test result to an acute endpoint and vice versa. For example, a chronic NOEC of 5 percent effluent (or $20 \mathrm{TU}_{\mathrm{C}}$ ) represents an acute $\mathrm{LC}_{50}$ of 50 percent (or $2 \mathrm{TU}_{\mathrm{a}}$ ) at an ACR of 10 .

EPA recommends that a discharger conduct chronic toxicity testing if the dilution of the effluent falls below 100:1 at the edge of the mixing zone. The rationale for this recommendation is that chronic toxicity has been observed in some effluents down to the 1.0 percent effect concentration. Therefore, chronic toxicity tests, although somewhat more expensive to conduct, should be used directly in order to make decisions about toxic impact.

There is a potential for acute toxicity within this dilution range, although this is less likely as the $100: 1$ dilution level is approached. Thus, the recommended screening protocol shown in Figure 3-2 includes a determination of whether excursions above the CMC are projected [4]. This analysis may be performed by assuming an ACR, applying this value to the chronic toxicity testing data, and allowing for any allowable initial mixing. Alternatively, the regulatory authority may use the interim results in the chronic test to calculate the acute toxicity.

Both the chronic and acute toxicity test data would be compared to their respective criterion. The chronic test results would be compared to the CCC, and the acute results, regardless of how calculated, would be compared to the CMC.

## Step 3: Decision Criteria for Permit Limit Development

Once the toxicity data have been generated for a discharger, the regulatory authority must decide whether or not the results show that the permittee causes, has the reasonable potential to cause, or contributes to an excursion of an applicable numeric or narrative water quality criterion and therefore needs to limit effluent toxicity. To do this, these data should be used to project receiving water concentrations, which are then compared to the CCC and CMC. One of four outcomes will be reached when following the screening protocol shown in Figure 3-2:

1) Excursion Above CMC or CCC - Where any one data point shows an excursion above the State's numeric or narrative criterion for the parameter toxicity, EPA regulations require a permit limit be set for whole effluent toxicity (40 CFR 122.44(d)(1)(iv or v)), unless limits on a specific chemical will allow the narrative water quality criterion to be attained or maintained. In the absence of a State numeric criterion for the parameter toxicity, EPA recommends that $1.0 \mathrm{TU}_{\mathbf{c}}$ and $0.3 \mathrm{TU}_{\mathrm{a}}$ be used as the CCC and CMC, respectively. The decision to develop permit limits based upon an excursion above either the CMC or CCC will lead to protection against both acute and chronic toxicity if the permit derivation procedures in Chapter 5 are used to set effluent limits.
2) Reasonable Potential for Excursion Above CMC or CCCEPA believes that "reasonable potential" is shown where an effluent is projected to cause an excursion above the CCC or CMC. This projection is based upon a statistical analysis of available data that accounts for limited sample size and effluent variability. EPA's detailed recommendations for making a statistical determination based upon effluent monitoring data alone are shown in Box 3-2. Where a regulatory authority finds that test results alone indicate a "reasonable potential" to cause an excursion above a State water quality criterion in accordance with 40 CFR 122.44(d)(1)(ii), a permit limit must be developed.

A regulatory authority may select an alternative approach for assessing reasonable potential. For example, an authority may opt to use a stochastic dilution model that incorporates both ambient dilution and effluent variability for determining reasonable potential. Such an approach is analogous to the statistical approach shown in Box 3-2. Whatever approach selected by the authority, it must use all the factors that account for all the factors listed in 40 CFR 122.44(d)(1)(ii).

In some cases the statistical analysis of the effluent data may not actually project an excursion above the CMC or CCC but may be close. Under such conditions, reasonable potential determinations will include an element of judgment on the part of the regulatory authority. Other factors will need to be considered and given appropriate weight in the decisionmaking process, including value of waterbody (e.g., high-use fishery), relative proximity to the CCC or CMC, existing controls on point and nonpoint sources, informa-
tion on effluent variability, compliance history of the facility, and type of treatment facility. These factors are summarized in Box 3-2 and are discussed in detail in Section 3.1. EPA recommends regulatory authorities establish a written policy and procedure for making determinations of "reasonable potential" under these circumstances.
3) No Reasonable Potential for Excursions Above CMC or CCC-In these situations, EPA recommends that the toxicity tests recommended above be repeated at a frequency of at least once every 5 years as a part of the permit application. Such testing is required for certain POTWs under 40 CFR 122.21 (j).
4) Inadequate information-Where a regulatory authority has inadequate information to determine reasonable potential for an excursion of a numeric or narrative water quality criterion, there may still be a basis for concern on the part of the authority. The permit should contain whole effluent toxicity monitoring requirements and a reopener clause. This clause would require reopening of the permit and establishment of a limit based upon any test results, or other new factors, which substantiate that the effluent causes, has the reasonable potential of causing, or contributes to an excursion above the CCC or CMC.

### 3.3.4 Use of Toxictly Testing in Multiple-Source Dlscharge Stuations

Where more than one discharge to the same receiving waterbody contributes, or has the reasonable potential to contribute to an excursion of water quality standards, permit limits must be developed for each individual discharger on that waterbody. For the regulatory authority to make this assessment, additional testing may be needed to provide the authority with the information necessary to assess the relative impact of each source. For purposes of this discussion, a multiple-source discharge situation is defined as a situation where impact zones overlap, or where ambient receiving water concentrations of a pollutant are elevated due to upstream discharges. In multiple-source discharge situations, additivity, antagonism, and persistence of toxicity can be of concern. To collect additional data, the permit authority should employ the toxicity testing procedures for multiple dischargers described in Box 3-3. In addition, ambient toxicity testing, as described below, could be used.

Assuming that screening has been conducted that reveals the need for permit limits, two options for controlling the discharges exist. The first option is for the permit authority to regulate each source separately using the procedures for individual point sources. In this option, the permitting authority would require use of upstream ambient water as a diluent in the toxicity test so as to be able to evaluate the contributions of upstream sources of toxicity. A second option is to treat each discharge as an interactive component of a whole system. In this option, the permit writer would determine a total maximum daily load for the receiving waterbody and develop individual wasteload allocations for each discharger using the procedures discussed in Chapter 4.
4) New information (other than revised regulations, guidance, or test methods) justifies relaxation of water quality-based permit limitations.
This last exception applies to water quality-based permit limitations only where the revised limitations result in a net reduction in pollutant loadings and are not the result of another discharger's elimination or substantial reduction of its discharge for reasons unrelated to water quality (e.g., operation termination).

Although Paragraph 402(o)(2) lists two additional exceptions, one for technical mistakes and mistakes of law and one for permit modifications or variances, the statute provides that these exceptions do not apply to water quality-based effluent limitations. As a result, these exceptions do not provide a basis for relaxing water quality-based limitations.

## Relaxation of Water Quality-Based Permit Conditions or Standards

The provisions in Section 402(o) discussed previously only address the relaxation of effluent limits based on water quality. The relaxation of other permit conditions or standards based on water quality are governed by EPA's existing antibacksliding regulations at 40 CFR $122.44(1)(1)$. Under these regulations when a permit is renewed or reissued, interim effluent limitations, standards, or conditions must be at least as stringent as the final effluent limitations, standards, or conditions in the previous permit "unless the circumstances on which the previous permit was based have materially and substantially changed since the time the permit was issued and would constitute cause for permit modification...". In other words, unless cause for permit modification is present, relaxed conditions or standards are not permissible. EPA regulations setting forth cause for permit modification can be found at 40 CFR 122.62.

## Restrictions of Backsliding

Even if any of the backsliding exceptions outlined in the statute or regulations are applicable and met, Section 402(o)(3) acts as a floor and restricts the extent to which water quality-based permit limitations may be relaxed. Paragraph (o)(3) prohibits the relaxation of water quality-based permit limitations in all cases if there will be a violation of applicable effluent limitation guidelines or water quality standards, including antidegradation requirements. This requirement affirms existing provisions of the CWA that require permit limits, standards, and conditions to ensure compliance with applicable technology-based limits and water quality standards.

### 5.8 TOXICTTY REDUCTION EVALUATIOMS

Where monitoring indicates unacceptable effluent toxicity, one principal mechanism for bringing a discharger into compliance with a water quality-based whole effluent toxicity requirement is a toxicity reduction evaluation (TRE) [6]. The purpose of a TRE is to investigate the causes and to identify corrective actions for difficult effluent toxicity problems. The permitting authority may require that the permittee conduct a TRE in those cases where the
discharger is unable to explain adequately and immediately correct exceedances of a whole effluent toxicity permit limit or requirement.

A TRE is a site-specific study conducted in a stepwise process to narrow the search for effective control measures for effluent toxicity. TREs are designed to identify the causative agents of effluent toxicity, isolate the sources of the toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity. The ultimate objective of a TRE is for the discharger to achieve the limits or permit requirements for effluent toxicity contained in the permit and thereby attain the water quality standards for receiving waters.

The requirement for a permittee to conduct a TRE may be written into the special conditions section of a permit, which contains whole effluent toxicity limits. In some cases, the permit issuing authority may also use other legally binding mechanisms, including Section 308 letters, Administrative Orders, or Consent Decrees, to require a TRE.

### 5.8.1 THE Guidance Documents

To assist permittees in conducting TREs and achieving compliance with whole effluent toxicity limits, EPA has developed a series of three guidance documents $[6,7,8]$ :

1) Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (EPA/600/2-88/070)
2) Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants (EPA/600/2-88/062)
3) Methods for Aquatic Toxicity Identification Evaluations:

Phase 1 Toxicity Characterization Procedures (EPA/600/388/034)
Phase 2 Toxicity Identification Procedures (EPA/600) 3-88/035)
Phase 3 Toxicity Confirmation Procedures (EPA/600/ 3-88/036).

These guidance documents describe the methods and procedures for conducting TREs and Toxicity Identification Evaluations (TIEs). They are based on the results of EPA's continuing efforts in TRE methods research and case study applications. Separate TRE guidance has been developed for industrial dischargers and municipal wastewater treatment plants to better address the circumstances of each type of facility. Procedures for the characterization, identification, and confirmation of the causative agents of effluent acute toxicity have been developed and are described in a three-phased TIE methods manual. These TIE methods are applicable to both industrial and municipal effluents and are an integral part of the protocols for TREs described in the industrial and municipal TRE quidance documents. TIE methods using chronic toxicity tests for identifying toxicants will soon be developed and available in a draft guidance document.

### 5.8.2 Recommonded Approach for Conducthy IRES

To ensure the successful completion of a TRE, the guidance documents recommend a systematic, stepwise approach that
eliminates the possible causes or sources of toxicity until a solution or control method is determined. The guidance documents discourage "playing hunches" or implementing extensive control measures solely on the basis of unsubstantiated conclusions (e.g., selecting and implementing a treatment plant upgrade without adequate information). Experience shows that unnecessary delays and expenditures in achieving the objective of the evaluation are avoided by building a sound scientific and engineering basis for selection of a control method. This can best be done by the logical interpretation of the information and data collected in a systematic approach to a TRE. The causes or control methods identified should then go through a confirmation stage. This is especially important in cases where the control method selected requires the construction of additional treatment. A flow chart, generalized from the guidance documents, for this approach to TREs is presented in Figure 5-10. The steps in this flow chart are summarized in the following discussion.

## Determination of TRE Objectives and Development of the TRE Plan

Obviously, the success of any study is dependent on a clear understanding of what is to be achieved and how these objectives are to be demonstrated and measured. Typically, TRE objectives are set by the regulatory authority in terms of a toxicity test endpoint (ATE or chronic toxicity endpoint [CTE]) in order to


Figure 5-10. Generalized TRE Flow Chart
meet a limit or permit condition. TRE plans should be submitted by the discharger as soon as possible. In some cases, this could be 30 to 60 days following notification that a TRE is required. In other instances, this period could be longer. These plans are important for ensuring that the TRE objectives are well understood and that the TRE to be conducted is thorough and represents a reasonable effort to achieve the required reduction in effluent toxicity. An implementation schedule should also be developed describing the timeframe for completion of the specific components of the TRE plan by the required TRE completion date. This schedule should be submitted for review in conjunction with the TRE plan. EPA recommends that the TRE schedule should be set or approved by the regulatory agency. Approval of the schedule and the completion date should not imply approval of the TRE plan itself or the procedures and methods outlined in the plan. Instead, the TRE plan should only be reviewed and any comments provided to the permittee as needed.

To assist in this review, Box 5-3 provides evaluation criteria for TRE plans. The permitting authority should review the TRE plan and inform the discharger of any apparent shortcomings or potential problems. The TRE should not be delayed pending completion of the review of the plan. The specified completion date for the TRE must still be met and the permittee should be expected to begin steps to investigate and alleviate the effluent toxicity as soon as possible following notification that a TRE is required. During the course of the TRE, the regulatory agency should provide oversight, as time permits, to make the TRE as effective as possible.

## Evaluation of Existing Site-specific Information

The next step involves the collection of any information and analytical data relevant to the effluent toxicity. The permittee should begin collecting and evaluating this information as soon as possible following notification that a TRE is required. In some cases, this step may be conducted concurrently with accelerated toxicity testing as part of the development of a TRE plan. For an industrial discharger, this part of the evaluation would include information such as plant and process information, influent and effluent physical and chemical monitoring data, effluent toxicity data, and material use. For a POTW, additional information, such as industrial waste survey applications, local limits compliance reports, and monitoring data, should be collected. This information is used to supplement the data generated in the later steps of the TRE and may be useful at that stage to point to potential sources or treatment options.

## Evaluation of Facility Operations and Maintenance Practices

This part of the evaluation is performed in order to ascertain whether the facility is consistently well operated and whether the effluent toxicity is the result of periodic treatment plant upsets, bypass, or some other operational deficiency that may be causing or contributing to the effluent toxicity. This part of the TRE should be initiated immediately after notification that a TRE is required. Alternatively, the permittee may begin to conduct this step at the same time that any accelerated toxicity testing is required. At both municipal and industrial facilities, this step would involve the evaluation of "housekeeping," treatment system operation, and chemical use. In some cases, best management practices (BMPs)
may be identified, which would improve operations and effluent quality. However, the effectiveness of BMPs in reducing effluent toxicity should be carefully confirmed, and it will usually be necessary to test a number of samples and perhaps to conduct Phase 1 of the TIE to develop this level of certainty. The results of this evaluation may lead to preliminary strategies for source reduction and pollution prevention, including spill or leak prevention, improvements in material handling and disposal practices, or substitution or re-use of a compound known to be highly toxic.

## Toxicity Identification Evaluation

TIE procedures are performed in three phases: characterization, identification, and confirmation [7]. In each phase, aquatic organism toxicity tests are used to track toxicity at each step of the procedure. In most cases, these are abbreviated or shortened toxicity tests. in the toxicity characterization phase, the general
nature of the causative agents of effluent toxicity or toxicants is determined. This is done by conducting a battery of tests to characterize the physical/chemical characteristics of the toxicity: solubility, volatility, decomposability, complexibility, filterability, and sorbability. This information can then be used to decide which chemical analytical methods will to use in Phase 2 or it can be used to design treatability studies.

The results of Phase 1 also may be used to provide additional confirmation of the effectiveness of any BMP that was implemented in the previous step of the TRE to reduce the effluent toxicity. This would require conducting at least one Phase 1 analysis prior to implementation of the BMP (i.e., any source control method implemented as a result of the evaluation of facility operation and maintenance). The results of this analysis would then be compared with Phase 1 results from samples taken after BMP implementation.

## Box 5-3. Evaluation Criteria for TRE Plans

- Are the objectives or targets of the TRE stated clearly and accurately?
- Are the schedule and milestones for accomplishing the tasks described in the study plan?
- Are the final TRE report, progress reports, and meetings with the regulatory authority included as part of the schedule?
- Are the approaches or methods to be used described to the extent possible prior to beginning the TRE?
- Has available EPA guidance been used in designing the TRE and developing the TRE plan (or if other methods are proposed, are these sufficiently documented)?
- Does the TRE plan specify what results and data are to be included in the interim and final reports?
- Does the TRE plan provide for arrangements for any inspections or visits to the facility or laboratory that are determined to be necessary by the regulatory authority?
- Are the toxicity test methods and endpoints to be used described or referenced?
- Does the approach described build on previous results and proceed by narrowing down the possibilities in a logical progression?
- Does the plan provide for all test results to be analyzed and used to focus on the most effective approach for any subsequent source investigations, treatability studies, and control method evaluations?
- Are optimization of existing plant/treatment operations and spill control programs part of the initial steps of the TRE?
- Does the TRE plan allow a sufficient amount of time and appropriate level of effort for each of the components of the study plan?
- Does the TIE use broad characterization steps and consider quantitative and qualitative effluent variability?
- Is toxicity tracked with aquatic organism toxicity tests throughout the analyses?
- Is the choice of toxicity tests for the TRE logical and will correlations be conducted if the species used are different from those used for routine biomonitoring?
- Is the laboratory analytical capability and the expertise of the investigator broad enough to conduct the various components of the evaluation?

In Phase 2 of the TIE, the results of Phase 1 are built upon, and the TIE proceeds to chemical analyses designed to identify the specific chemicals causing effluent toxicity. In Phase 3, the identified toxicants are confirmed using a number of procedures, including correlation of toxicity with chemical concentration, spiking experiments, toxicity mass balance, and additional test species and their symptoms.

The current version of the TIE methods uses acute toxicity tests to characterize and identify the toxicants. In some cases, these methods may also be used for TREs where the objective is to reduce chronic toxicity. In order for these methods to be applicable, however, there must be some measurable acute toxicity in the effluent samples that are to be characterized in Phase 1 and analyzed in Phase 2. If this approach is used, the appropriate chronic toxicity test, as specified in the TRE objectives and permit requirements, should then be used in the Phase 3 confirmation procedures. This will confirm that the toxicant(s) identified using acute tests in Phases 1 and 2, are indeed causing the whole effluent chronic toxicity, which must be reduced.

It is possible to use the methods and procedures described in the other components of the overall TRE with either acute or chronic toxicity tests. The fact that the previous version of the EPA TIE methods use acute toxicity tests should not be construed to mean that TREs cannot be required or conducted for the reduction of chronic toxicity. These methods provide additional tools to assist permittees in the reduction of whole effluent chronic toxicity. Phase 1 procedures that use chronic toxicity tests will soon be available in draft EPA guidance. These TIE methods are applicable to freshwater discharges to either saltwater or freshwater receiving waters. The use of these methods for saltwater receiving waters may require their adaption for use with marine test species or, preferably, an initial correlation of the recommended freshwater TIE test species to the marine species used for monitoring.

## Source Investigation

Based on the results of the TIE, a decision is made on whether to conduct treatability studies on the final effluent and/or conduct a source investigation. A source investigation is most readily performed when the specific toxicants have been identified and influent samples can be analyzed for the presence of these compounds or when potential source streams can be selected for chemical analysis (based on the results of the initial data acquisition step). However, in some cases where the specific causative agents of effluent toxicity have not been identified in the TIE, it may be possible to conduct a source investigation by "treating" influent samples in bench-scale models of the facility treatment plant, measuring the toxicity of the treated sample and then tracking this toxicity to its source.

Source investigations will lead to control methods, such as chemical substitution, process modification, treatment of process or influent streams (pretreatment), and possible elimination of the process. For POTWs, source investigations may lead to the development of local limits or to the requirement that an indirect discharger evaluate and control their effluent so as to reduce its toxicity and prevent passthrough at the POTW. The implementation of source control methods can effectively reduce effluent toxicity and also can avoid any cross-media transfer of pollutants
to air or sludge, which may occur as a result of end of pipe treatment. Types of source control methods that have proven to be effective in reducing effluent toxicity are improvements in facility housekeeping, chemical substitution, process optimization, reclamation/re-use, and pretreatment.

## Toxicity Treatability Evaluation

Toxicity treatability evaluations are conducted to identify possible treatment methods that can effectively reduce effluent toxicity and may involve modifications or additions to the existing system. Treatability studies generally use the same type of information on the nature of the chemicals to be removed as is generated by Phase 1 of the TIE. These treatability tests should be conducted on a bench-scale initially and then a pilot scale prior to construction of additional treatment or substantial modification of the existing plant. The use of these bench- and pilot-scale tests, coupled with aquatic organism toxicity tests, should be used to confirm the effectiveness of the treatment option. Confirmation of the results of treatability studies is equally important as it is for the TIE. Skipping this confirmation step is an invitation for unwarranted expense.

## Toxicity Control Method Selection and Implementation

After the investigative steps of the TRE are completed, it is not unusual for a number of possible control options to have been identified. At this point, a site specific selection must be made by the discharger based on the technical and economic feasibility of the various alternatives. Following this selection, the toxicity control method is implemented or a compliance plan is submitted if construction of additional treatment requires a substantial amount of time.

## Followup and Confirmation

After the control method is implemented and the final TRE report is submitted, the permitting agency should direct the permittee to conduct followup monitoring to confirm that the reduction in effluent toxicity is attained and maintained. Normally, this monitoring should follow an accelerated schedule, weekly or biweekly toxicity tests, for a period of 2 to 3 months to confirm the effectiveness of the controls implemented and the continued attainment of the TRE objective. This followup monitoring should use the same species as were specified for routine toxicity testing in the permit. The test endpoints of these toxicity tests should be the same as those which were calculated by the water qualitybased permit limit derivation procedure used when the permit was issued. Once the discharger has demonstrated the successful completion of the TRE, the permitting agency should direct the discharger to return to the routine permit monitoring schedule.

### 5.8.3 Circumstances Warranting a TRE

It is the responsibility of the permitting authority to determine if the permit limits and/or the State water quality criteria have been threatened or violated and to notify the permittee if a TRE is required. It is appropriate for the permitting authority to require additional toxicity testing following the initial exceedance or violation. This additional testing may precede notification that a TRE will be required or it may be considered as the initial part of the

TRE and be conducted simultaneously with TRE plan development and the evaluation of other existing site-specific information.

It is important to recognize that the purpose of this additional toxicity testing is to determine the continued presence or absence of effluent toxicity and the magnitude of that toxicity. This information can then be used to determine the continued compliance or noncompliance with the limit or permit conditions for effluent toxicity. These tests do not serve to verify or confirm the initial test results from an earlier sample. Instead, the permit authority shall use the results of these tests to determine if a TRE or some other action is the appropriate response to the initial occurrence of toxicity.

If the permit has a limit for whole effluent toxicity, then generally, the permit should not include any specific conditions for accelerated toxicity testing or for triggering a TRE or some other action (e.g., exceedances in two consecutive tests or exceedances in any three out of five tests). CWA Section 309 requires that any single violation of a permit limit may be subject to enforcement. The EPA Compliance Monitoring and Enforcement Strategy for Toxics Control (January 19, 1989, Appendix B-4) states that, "Each exceedance of a directly enforceable whole effluent toxicity limit is of concern to the regulatory agency and therefore qualifies as meeting the VRAC [violation review action criterion] requiring professional review." Accelerated monitoring should only be used to assist in this professional review to determine what, if any, enforcement response is necessary, including the need for the permittee to conduct a TRE. It will be necessary for the Region or State regulatory authority to determine this on a case-by-case basis. This must be done in a manner consistent with the priorities established in their respective toxics control strategies and permitting procedures.

In situations where it is determined that accelerated testing is appropriate, a maximum of weekly tests for a minimum period of 2 months is recommended. This would result in eight tests, plus the routine monitoring toxicity test that initially indicated the exceedence or violation, for a total of nine tests in the series. As a practical approach for determining if a TRE is an appropriate response, EPA recommends if toxicity is repeatedly or periodically present at levels above the effluent limits more than 20 percent of the time, a TRE should be required. With toxicity present at this rate, the TRE protocols will be useful.

In most cases, any one additional exceedance (beyond the initial routine monitoring toxicity test result) in the accelerated toxicity tests could result in notification of the permittee that a TRE is required. Exceptions to this guideline might include cases where the permittee is able to adequately demonstrate that the cause of the exceedances is known and corrective actions have been immediately implemented or cases where additional test quality assurance/quality control (QA/QC) is necessary or desirable. The submittal of QC fact sheets for self-biomonitoring (e.g., Appendix B-2) should always be recommended to avoid QA/QC problems.

If the test results indicate that toxicity is not consistently or repeatedly present in the test series, previous discharge monitoring reports (DMRs) should be examined to ascertain if a recurrent problem exists. If the problem is recurrent, a TRE should be required, and the TRE plan should explain how the design of the
evaluation will address this periodic or recurrent effluent toxicity problem. In these cases, more elaborate sampling design and influent or process stream monitoring may be needed. It should be expected that TREs conducted under these circumstances will probably require a more flexible schedule and perhaps additional time before the required completion date.

If the accelerated testing and previous DMRs show the continued absence of effluent toxicity, then the initial exceedance would be considered an episodic event and a TRE should not be required. A TRE is not an appropriate response to a single, episodic effluent toxicity event (e.g., a spill or a plant upset). By conducting accelerated testing following a violation or exceedance of a permit condition, unnecessary TREs can be avoided. Similarly, conducting accelerated testing as part of the initial steps of a TRE will allow for the TRE to be ended in its very early stages if the toxicity is immediately controlled or determined to be episodic or nonrecurrent. By following the TRE guidance and incorporating accelerated testing into the TRE, unnecessary analyses and expense can be avoided.

It also is important to note that for the practical purposes of conducting a TRE (as opposed to the purpose of determining if a TRE should be required or not), the magnitude of the effluent toxicity needed to conduct a TRE may be less than the magnitude or level set as the permit limit or permit monitoring condition. This is because if the limit or monitoring condition is water quality-based then some amount of dilution will usually be incorporated in determining the unacceptable level of effluent toxicity. In some cases, it may be possible for the TRE procedures to be carried out even if the toxicity does not actually exceed this permitted level. This will be the case as long as the effluent toxicity is periodically or consistently present in measurable amounts in samples of 100 -percent effluent.

It also is reasonable for a discharger to initiate a TRE prior to the establishment of a permit limit for toxicity if unacceptable levels of toxicity are found in the effluent through routine monitoring or through inspection and compliance sampling by the regulatory authority. Under these circumstances the regulatory authority will need to identify what constitutes unacceptable levels of toxicity since this will not be defined by a permit limit (see Chapter 3 on determining the reasonable potential for excursions of water quality standards). It also is not unreasonable for the discharger to voluntarily initiate a TRE under these circumstances.

### 5.8.4 Mochanlsms for Requising TREs

There are a number of mechanisms that can be used to require a TRE. In most cases, the TRE should be required by a Section 308 letter or by an enforcement action, such as a Section 309 Administrative Order or a Consent Decree. The permittee should receive notification from the permit authority of what response is required. This enables the permit authority to assess whether a TRE is the appropriate action to pursue. If effluent toxicity reappears following the successful completion of a TRE, then the permit authority should be able to review this type of situation to determine if an additional TRE is appropriate or if some other action is required. In general, when the permit is issued with whole effluent toxicity limits in Part 1 of the permit, TRE requirements should be used where necessary to bring the permittee into compliance with those limits. Box 5-4 provides example lan-

