tested quarterly for a minimum of one year. For multiple species, EPA requires a minimum of two species (e.g., vertebrates and invertebrates). The permitting authority may require the applicant to include other species (e.g., plants) as well. Applicants must provide these tests for acute or chronic toxicity, depending on the range of the receiving water dilution. EPA recommends that applicants conduct acute or chronic toxicity testing based on the following dilutions:

- Acute toxicity testing if the dilution of the effluent is greater than 1000:1 at the edge of the mixing zone.
- Acute or chronic toxicity testing if the dilution of the effluent is between 100:1 and 1000:1 at the edge of the mixing zone. Acute testing may be more appropriate at the higher end of this range (1000:1), and chronic testing may be more appropriate at the lower end of this range (100:1).
- Chronic toxicity testing if the dilution of the effluent is less than 100:1 at the edge of the mixing zone.

All data provided in Part B must be based on tests performed within three years prior to completing this application. The tests must have been conducted since the last NPDES permit issuance or permit modification under 40 CFR 122.62(a). In addition, applicants only need to submit data that have not previously been submitted to the permitting authority. Thus, if test data have already been submitted (within the last three years) in accordance with an issued NPDES permit, the treatment works may note the dates the tests were submitted and need not fill out the information requested in question B.2. for that test.

Additional copies of Part B may be used in submitting the required information. A permittee having no significant toxicity in the effluent over the past year and who has submitted all toxicity test results through the end of the calendar quarter preceding the time of permit application would need to supply no additional data as toxicity testing data as part of this application. Instead, the applicant should complete question B.4., which requests a summary of bioassay test information already submitted. (See below for more detailed instructions on completing question B.4.)

Where test data are requested to be reported, the treatment works has the option of reporting the requested data on Form 2A or on reports supplied by the laboratories conducting the testing, provided the data requested are complete and presented in a logical fashion. The permitting authority reserves the right to request that the data be reported on Form 2A.

B.1. Required Tests

a. Provide the total number of chronic and acute whole effluent toxicity tests conducted in the past three years. A ''chronic'' toxicity test continues for a relatively long period of time, often one-tenth the life span of the organism or more. An ''acute'' toxicity test is one in which the effect is observed in 96 hours or less.

B.2. Individual Test Data

Complete B.2. for each test conducted in the last three years for which data has not been submitted. Use the columns provided on the form for each test and specify the test number at the top of each column. Use additional copies of question B.2. if more than three tests are being reported. The parameters listed on the form are based on EPA-recommended test methods. Permittees may be required by the permitting authority to submit additional test parameter data for the purposes of quality assurance.

If the treatment works is conducting whole effluent toxicity tests and reporting its results in accordance with an NPDES permit requirement, then the treatment works may note the dates the tests were submitted and need not fill out the information requested in question B.2. for those tests (unless otherwise required by the permitting authority).

a. Provide the information requested on the form for each test reported. Under ''Test species,'' provide the scientific name of the organism used in the test. The ''Outfall number'' reported must correlate to the outfall numbers listed in questions 15–17 of the Basic Application Information packet.

b. Provide the source of the toxicity test methods followed. In conducting the tests, the treatment works must use methods approved in accordance with 40 CFR Part 136 [Note: Approved methods are currently under development].

c. Indicate whether 24-hour composite or grab samples were used for each test. For multiple grab samples, provide the number of grab samples used. Refer to Appendix A of the instructions for a definition of composite and grab samples.

d. Indicate whether the sample was taken before or after disinfection and/or after dechlorination.

e. Provide a description of the point in the treatment process at which the sample was collected.

f. Indicate whether the test was intended to assess chronic or acute toxicity.

g. Indicate which type of test was performed. A ''static'' test is a test performed with a single constant volume of water. In a ''static-renewal'' test, the volume of water is renewed at discrete intervals. In a ''flow-through'' test, the volume of water is renewed continuously.

h. Indicate whether laboratory water or the receiving water of the tested outfall was used as the source of dilution water. If laboratory water was used, provide the type of water used.

i. Indicate whether fresh or salt water was used as the dilution water. For salt water, specify whether the salt water was natural or artificial (specify the type of artificial water used).

j. For each concentration in the test series, provide the percentage of effluent used.

k. Provide the minimum and maximum parameters measured during the test for pH, salinity, temperature, ammonia, and dissolved oxygen.

l. Provide the results of each test performed. For acute toxicity tests, provide the percent survival of the test species in 100 percent effluent. Also provide the $LC_{50}$ (Lethal Concentration to 50 percent) of the test. ''$LC_{50}$'' is the effluent (or toxicant) concentration estimated to be lethal to 50 percent of the test organisms during a specific period. Indicate any other test results in the space provided.

For chronic toxicity tests, provide data at the most sensitive endpoint. While this is generally expressed as a ''NOEC'' (No Observed Effect Concentration), it may be expressed as an ''Inhibition Concentration'' (e.g., ''$IC_{25}$''—Inhibition Concentration to 25 percent). The NOEC is the highest measured concentration of an effluent (or a toxicant) at which no significant adverse effects are observed on the test organisms at a specific time of observation. The $IC_{25}$ is the effluent (or toxicant) concentration estimated to cause a 25 percent reduction in reproduction, fecundity, growth, or other non-quantal biological measurements. Indicate any other test results in the space provided.

m. Provide the mortality (in percent) of the control group. Indicate any other relevant information about the control group in the space provided.

B.3. Toxicity Reduction Evaluation

A Toxicity Reduction Evaluation (TRE) is a site-specific study conducted in a stepwise process designed to identify the causative agents of effluent toxicity, evaluate the effectiveness of