Stormwater Toxicity

The water quality of stormwater, or of ambient waters immediately following high flow events, has been shown to be degraded in many studies with chemical concentrations which may exceed toxicity thresholds (e.g., Horner et al. 1994; Makepeace et al. 1995; Morrison et al. 1993; Waller et al. 1995a). Stormwater toxicants are primarily associated with particulate fractions and are typically assumed to be “unavailable.” Toxicity tests with sediment removed have found reduced levels of toxicity in stormwater, compared to stormwater that has not undergone sediment removal (Crunkilton et al. 1996), as described in Chapter 3.

Also confusing is that typically short and intermittent runoff events cannot be easily compared to the criteria or standards developed and tested for traditional “long” duration point source discharges. Chemical analyses, without biological analyses, typically underestimate the severity of the problems because the water column quality varies rapidly, while the major problems were associated with sediment quality and effects on macroinvertebrates (Lenat and Eagleson 1981; Lenat et al. 1981).

Standardized toxicity tests have been used for many years in the United States to evaluate effluents in the National Pollutant Discharge Elimination System (NPDES) (EPA 1991e). These
whole-effluent toxicity (WET) tests have been shown to be useful for evaluating stormwaters. The use of toxicity tests on stormwater and receiving waters, especially in situ and side-stream tests that also reflect changing conditions for extended periods, has added greatly to our knowledge of toxicant problems associated with stormwater. While some stormwaters may not be toxic, there is a large body of evidence that suggests many are. Laboratory testing of runoff samples has shown acute and chronic toxicity to a variety of species (Connor 1995; Cooke et al. 1995; Dickerson et al. 1996; Hatch and Burton 1999; Ireland et al. 1996; Katzenelson et al. 1995; Kuivila and Foe 1995; McCahon and Pascoe 1990, 1991; McCahon et al., 1990, 1991; Medeiros and Coler 1982; Medeiros et al. 1984; Mote Marine Laboratory 1984; Tucker and Burton 1999; Werner et al. 2000; Vlaming et al. 2000; Bailey et al. 2000). Pesticide pulses have been followed through watersheds, remaining toxic for days to weeks from runoff (Kuivila and Foe 1995; Werner et al. 2000). Samples from urban streams in southern California showed 85% exceeded diazinon criteria and 80% exceeded chlorpyrifos criteria. Of these samples, 76.6% produced 100% C. dubia mortality within 72 hours of exposure. Toxicity Identification Evaluations (TIE) confirmed the toxicity was due to the pesticides. Diazinon has been implicated as the primary toxicant in runoff causing acute toxicity to C. dubia, P. promelas, and in situ Corbicula fluminea assays (Kuivila and Foe 1995; Connor 1995; Waller et al. 1995a,b; Cooke et al. 1995). Organophosphate (chlorpyrifos, diazinon, malathion) and carbamate (carbofuran, carbaryl) pesticides in a delta draining urban and agricultural areas were the primary toxicants causing acute toxicity in 9.8 to 19.6% of water samples sampled between 1993 and 1995 (Werner et al. 2000). C. dubia reproduction and growth of C. fluminea in situ closely paralleled the health of the indigenous communities (Dickson et al. 1992; Waller et al. 1995b). A simulation of farm waste effluent (increased ammonia and reduced dissolved oxygen) found amphipod precopula disruption to be the most sensitive indicator of stress (McCahon et al. 1991). Mortality occurred only when D.O. fell to 1 to 2 mg/L and feeding rates recovered after exposure to ammonia (5 to 7 mg/L) ended. Elevations of major ion concentrations were toxic to C. dubia and P. promelas in some irrigation drainage waters (Dickerson et al. 1996).

Toxicity may also be reduced in runoff. When turbidity increased during high flow, photoinduced toxicity of PAHs was reduced in situ, as compared to baseflow conditions (Ireland et al. 1996). A recent study of the chronic toxicity of fenoxycarb to Daphnia magna showed a realistic single-pulse exposure resulted in an MATC of 26 µg/L, as compared to 0.0016 µg/L from a standard, constant-exposure study (Hosmer et al. 1998).

WET tests have also been used to evaluate the toxicity of effluents from stormwater runoff treatment systems. An evaluation of an urban runoff treatment marsh found strong relationships between C. dubia time-to-death, conductivity, and storm size, and time from storm flow initiation (Katzenelson et al. 1995). Airport runoff containing glycol-based deicer/anti-icer mixtures was toxic to P. promelas and D. magna during high-use winter months; however, during summer months runoff toxicity only coincided with fuel spills (Fisher et al. 1995). Anti-icer was more toxic to P. promelas, D. magna, D. pulex, and C. dubia than deicer. Additives were more toxic than glycols (Hartwell et al. 1995). Stormwater detention ponds reduced P. promelas and Microtox toxicity 50 to 90% when particles greater than 5 µm were removed (Crunkilton et al. 1996; Pitt et al. 1999a).

**Pulse Exposures**

Some have suggested that relatively short periods of exposure to the toxicant concentrations in stormwater are not sufficient to produce the receiving water effects that are evident in urban receiving waters, especially considering the relatively large portion of the toxicants that are associated with particulates (Lee and Jones-Lee 1995a,b). Lee and Jones-Lee (1995b) suggest that the biological problems evident in urban receiving waters are mostly associated with illegal discharges and that the sediment-bound toxicants are of little risk. Mancini and Plummer (1986) have long been advocates of numeric water quality standards for stormwater that reflect the partitioning of the toxicants and the short periods of exposure during rains. Unfortunately, this approach attempts
to isolate individual runoff events and does not consider the cumulative adverse effects caused by the frequent exposures of receiving water organisms to stormwater (Davies 1986, 1991, 1995; Herricks 1995; Herricks et al. 1996).

A growing preponderance of data, however, is showing that toxicity is commonly observed during stormwater runoff and that short-term, pulse exposures can be more toxic than long-term, continuous exposures (e.g., Brent and Herricks 1998; Crunkilton et al. 1996; Curtis et al. 1985). Short pulse exposures in stormwater produced lethality several days to weeks later (Abel 1980; Bascombe 1988; Bascombe et al. 1989; Brent and Herricks 1998; Ellis et al. 1992). Some of this apparent response delay may be a result of uptake and accumulation kinetics (Bascombe et al. 1989, 1990; Borgmann and Norwood 1995; Borgmann et al. 1993). Recent investigations have identified acute toxicity problems and the importance of an adequate post-exposure observation period in side-stream studies with P. promelas in urban streams (Crunkilton et al. 1996), and in laboratory spiking studies (Cd, Zn, phenol) with Ceriodaphnia dubia, Pimephales promelas, and Hyalella azteca (Brent and Herricks 1998; Van Der Hoeven and Gerritsen 1997). Other laboratory studies have also shown acute and chronic toxicity of short-term exposures using fish and amphipods exposed to chloroamines, metals, and pesticides (Abel 1980; Abel and Gardner 1986; Holdway et al. 1994; Jarvinen et al. 1988a,b; McCahon and Pascoe 1991; Meyer et al. 1995; Parsons and Surgeoner 1991a,b; Pascoe and Shazili 1986). In general, it appears that exposure to higher concentrations of toxicants for brief periods is more important than exposure to lower concentrations for longer periods (Brent and Herricks 1998; McCahon and Pascoe 1990; Meyer et al. 1995). However, increased amphipod depuration or metallothionein induction in the presence of Zn allowed greater tolerance (Borgmann and Norwood 1995; Brent and Herricks 1998).

Not all pulsed exposures are more toxic. If there is adequate time for organism recovery between pulsed exposures to toxicants, the effects of the pulsed exposure of some toxicants are diminished (Brent and Herricks 1998; Kallander et al. 1997; Mancini 1983; Wang and Hanson 1985). This difference may be attributed to the mechanism of toxicity. For example, organophosphates are relatively irreversible inhibitors of acetylcholinesterase (AChE), while carbamate inhibition may be reversible (Kuhr and Dorough 1976; Matsumura 1985). So little difference is observed between continual exposures and pulsed exposures (Kallander et al. 1997). Trout were observed to acclimate to ammonia if pulsed exposures were below their toxicity threshold (Thurston et al. 1981). Fenoxycarb was four orders of magnitude less toxic in a single pulsed exposure to Daphnia magna compared to a standard WET exposure (Hosmer et al. 1998). Complicating predictions of effects are synergistic interactions that occur between some contaminants such as pesticides and metals (Forget et al. 1999) and between herbicides and insecticides (Pape-Lindstrom and Lydy 1997). Organisms recovered to varying degrees given adequate time in clean water following pulsed exposures to phenol, permethrin, fenitrothion, and carbamates (Brent and Herricks 1998; Green et al. 1988; Kallander et al. 1997; Kuhr and Dorough 1976; Parsons and Surgeoner 1991a,b).

**Measuring Effects of Toxicant Mixtures in Organisms**

Toxicant exposure is dependent on toxicant, organism, and habitat characteristics, such as toxicant partitioning (fugacity), the organisms’ direct contact with substrates, and their feeding mechanisms. The toxicant target site and effect within the organism will be toxicant, species, and life stage dependent. The mixed function oxygenase (MFO) system and metallothionein production are well-known metabolic processes which often detoxify compounds, converting them to excretable metabolites (Rand and Petrocelli 1985). These metabolic systems vary dramatically among aquatic species, so it is difficult to predict aquatic toxicity to multiple species without actual testing each species. All of the above uncertainties associated with toxicant differences and interactions, exposure pathways, and organism responses support the use of multiple species in stormwater assessments.

There are mixtures of chemicals in stormwaters. Since chemical water quality criteria and standards only consider effects from one chemical, the question arises as to what effects may result
to organisms when they are exposed to a mixture of potentially toxic chemicals. Mixture effects have been studied for decades. Sprague and Ramsay (1965) proposed a toxic unit (TU) that defined the strength of a toxicant. One toxic unit is equal to the incipient LC50 (the level of a toxicant that is lethal to 50% of the individuals exposed for a period of time where acute lethal effects have ceased). The strength of a toxicant, or the TU, is calculated as actual toxicant concentration in solution divided by the LC50. If the calculated sum of toxic units in a mixture of chemicals is one or larger, the mixture is said to be lethal.

The EPA (1991e) assumes that chemical toxicants act in an additive fashion, as opposed to being antagonistic (less toxicity than predicted) or synergistic (greater toxicity than predicted). A great deal of experimentation has been completed in this area, and some general principles have emerged. Overall, it appears that joint toxicity often occurs among chemicals with a similar mode of action. Within similar modes of action, the concentration-addition model (often called the TU concept) often describes the interaction

\[
\text{TU mixture} = \sum_{i=1}^{n} \text{TU}_i
\]

Additivity or near additivity has been demonstrated for many groups of chemicals, such as narcotics, organophosphate pesticides, pyrethroid pesticides, polynuclear aromatic hydrocarbons, major ions, and metals (Sprague 1968; Sprague and Ramsay 1965; Broderius and Kahl 1985; Carder and Hoagland 1998; Deneer et al. 1988a; Hermens and Leewaugh 1982; Hermens et al. 1984a,b,c; Konemann 1981; Muska and Weber 1977).

In contrast to mixtures of chemicals with similar modes of action, chemicals with dissimilar modes of action (e.g., zinc and diazinon) show antagonistic, little, or no interaction, such that the toxicity of a binary mixture shows toxicity equal to or less than that of the most toxic component (Howell 1985; Herbes and Beauchamp 1977; Schultz and Allison 1979; Deneer et al. 1988b; Spehar and Fianlt 1986; Alabaster and Loyd 1982).

Extreme interactions of chemical mixtures, such as synergy (TU mixture >> \( \sum \text{TU}_i \)) have also been frequently reported (Sprague and Ramsay 1965; Spehar and Fianlt 1986; Sharma et al. 1999; Christensen 1984; Vasseur et al. 1988; Marking 1977; Christen 1999; Marking and Dawson 1975; Anderson and Weber 1977; Doudoroff 1952; Wink 1990; Pape-Linstrom and Lydy 1997; Forget et al. 1999). One mechanism for synergism is where one chemical has a potentiating effect on the physiological pathway that is the target of a second toxicant. The classic example is piperonyl butoxide and pyrethroid pesticides; piperonyl butoxide blocks the detoxification pathway for pyrethroids, thereby greatly exacerbating their toxicity. In fact, this interaction is used intentionally in pyrethroid pesticide formulations.

While laboratory experiments have demonstrated approaches for mixture assessment, the test of the approach lies in its effectiveness when applied to mixtures occurring in the field, and experience suggests that the approach of assuming addition within modes of action and independence between different modes of action is adequate in many cases. For example, in studies of over 80 municipal and industrial effluents, toxicity identification studies showed no instances where observed toxicity was greater than would be predicted by this approach (D.R. Mount and J.R. Hockett, unpublished data).

The finding that mixture models are necessary to account for the potency of PAHs and dioxin-like compounds in the field provides excellent insights into the circumstances necessary for the expression of interactive toxicity in the environment. In addition to sharing a common mode of action (narcosis for PAHs; Ah-receptor agonism for dioxins/furans/PCBs), the sources for these contaminants and their environmental fate are such that they occur in mixture compositions where multiple components contribute meaningfully to the toxicity. The absence of the latter attribute greatly simplifies the assessment of many mixtures. In cases where one component of the mixture
dominates, ignoring toxic interactions within the mixture adds little uncertainty to the overall assessment. Metals provide an excellent example. In practice, many metal mixtures are dominated by a particular metal. Hence, assessing the potency of the mixture on the basis of its most potent component is often effective. In the case of PAHs, however, multiple individual PAHs contribute substantially to toxicity, and the additive toxicity must be taken into account to adequately assess the mixture.

Unfortunately, the many studies cited above suggest that toxicity resulting from stressor mixtures cannot be accurately predicted simply based on additivity or chemical type. A number of studies have shown that interactions of chemical mixtures can change from antagonistic to synergistic based on the life stage of the organisms, concentrations or levels of the contaminants, or length of exposures (Sprague and Ramsay 1965; Eaton 1973; Spehar and Fiaudt 1986; Marking and Dawson 1975; Munawar et al. 1987; Sharma et al. 1999; Cairns et al. 1978). This suggests that site-specific in situ assessments of toxicity and biological communities, as discussed later in this section, are necessary for establishing the effects of stormwater runoff.

**Standard Testing Protocols: Waters**

As with any of the preceding assessment methods and approaches, it is usually important that standard methods and proper QA/QC practices be followed. This helps ensure the production of valid data that are comparable to other similar study results, are reproducible, and may be usable in enforcement actions. For many of the toxicity test applications, standard methods exist, either as EPA, state, APHA, or ASTM methods. However, the absence of a standard method, such as for in situ or multispecies assays, does not preclude their use. These “nonstandard” assays should be based on methods published in peer-reviewed scientific periodicals that have been demonstrated as valid and useful. Since this science is relatively young, the standardization process is also young and ongoing. Standard test species have been shown to represent the sensitive range of ecosystems analyzed (EPA 1991e). In addition, resident species testing is more difficult and subject to variability than standardized testing, and many important quality assurance–quality control requirements (e.g., same life stage, sensitive life stage, reference toxicant testing, interlaboratory variation, acclimation) cannot be met (EPA 1991e).

The preferred assessment design is to have toxicity tests as a screening and definitive tool, using acute and short-term chronic toxicity measures from multiple levels of biological organization. This approach has been the foundation of chemical-specific water quality criteria development and modification. Most toxicity test requirements in NPDES permits require the use of the fathead minnow (*Pimephales promelas*) and cladoceran *Ceriodaphnia dubia* (Figures 6.141 through 6.143). However, the EPA recommends that three species be tested in whole-effluent toxicity (WET) calculations including a fish, an invertebrate, and an algae (EPA 1991e). EPA guidance on hazardous waste site evaluations suggested the fish, *Daphnia*, and green algal (*Selenastrum capricornutum*) assays (Figure 6.144), along with terrestrial testing of seed germination and root elongation,
earthworm survival, and soil respiration (Table 6.55; EPA 1989a,b; Porcella 1983). The ASTM and EPA now have standardized methods for sediment toxicity and bioaccumulation evaluations using benthic macroinvertebrates (EPA 2000c; ASTM 2000). They recommend a multispecies approach that is essential, as no one organism can serve as a surrogate for all species. An analysis of species sensitivity ranges observed in the National Water Quality Criteria documents found that when four or more species were tested, the LC50 of all was within one order of magnitude for 71 of the 73 pollutants tested (EPA 1991e). No one species was consistently the most sensitive (EPA 1991e).

A wide variety of useful and sensitive assays exists for toxicity evaluations of waters (Table 6.56) and sediments (Table 6.57). The optimal assay(s) is dependent on several issues, which will vary with the geographic area, study objectives, and pollutant problem (Table 6.58). For typical stormwater assessments, a tiered assessment approach is warranted, where the initial runoff is tested using a toxicity screening technique using the water flea (D. magna, D. pulex, or C. dubia) in 24- to 48-hour exposures. Additionally, if depositional (clay-silt) sediments exist downstream of stormwater outfalls, they should be evaluated for toxicity using EPA 10-day whole-sediment methods. If no toxicity is detected, however, the community indices of the benthic macroinvertebrate or fish communities indicate impairment, additional toxicity testing should be conducted, such as short-term chronic toxicity (EPA 7-day assays) and/or in situ toxicity exposures (described below and Appendix D). If toxicity problems are identified in the stormwater samples from the screening tests, definitive testing is conducted that may consist of acute to chronic laboratory, on-site, and/or in situ exposures; testing whole sediment, ambient water, or effluent; testing additional species such as bacteria (e.g., Microtox), photosynthetic organisms (e.g., duckweed, green algae), and fish (e.g., fathead minnow); and/or TIE evaluations to identify specific toxicants.

Defining stormwater toxicity at both a spatial and temporal scale may require large numbers of samples, which would surpass the resource capabilities of most projects if attempting to run conventional EPA-approved surrogate species (e.g., P. promelas and C. dubia). Stressor variability, as discussed previously, will be substantial through the course of a storm event and the return to baseflow conditions. The EPA recommends that for sampling of effluents and for annual monitoring of effluents using grab sampling, a minimum of four to six samples be collected in 1 day, once per month, to better define short-term variation. Sewage treatment plant effluents typically have shown coefficients of variation (COV) for acute toxicity of 20 to 42% and 0 to 88% for chronic toxicity. Among oil refinery effluents, the COVs ranged from 19 to 54% for acute and 30 to 60% for chronic data. Other manufacturing facility effluents had acute toxicity COVs of 20 to 100% (EPA 1991e). It may be useful to split definitive samples and run Microtox in tandem with the macrofaunal assays. If a consistent relationship is observed, i.e., few false positive or false negatives using
### Table 6.55 Toxicity Evaluation Categories for Hazardous Waste Sites

<table>
<thead>
<tr>
<th>Assay</th>
<th>Activity Measured</th>
<th>Sample Type</th>
<th>MAD</th>
<th>Units</th>
<th>High</th>
<th>Moderate</th>
<th>Low or Not Detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater fish</td>
<td>96-hr LC50 (lethality)</td>
<td>S</td>
<td>1</td>
<td>g/L</td>
<td>&lt;0.01</td>
<td>0.01–0.1</td>
<td>0.1–1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>100</td>
<td>%</td>
<td>&lt;20</td>
<td>20–75</td>
<td>75–100</td>
</tr>
<tr>
<td>Freshwater invertebrate</td>
<td>46-hr EC50 (immobilization)</td>
<td>S</td>
<td>1</td>
<td>g/L</td>
<td>&lt;0.01</td>
<td>0.01–0.1</td>
<td>0.1–1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>100</td>
<td>%</td>
<td>&lt;20</td>
<td>20.75</td>
<td>75–100</td>
</tr>
<tr>
<td>Freshwater algae</td>
<td>96-hr EC50 (growth inhibition)</td>
<td>S</td>
<td>1</td>
<td>g/L</td>
<td>&lt;0.01</td>
<td>0.1–0.1</td>
<td>0.1–1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>100</td>
<td>%</td>
<td>&lt;20</td>
<td>20–72</td>
<td>75–100</td>
</tr>
<tr>
<td>Seed germination and</td>
<td>115-hr EC50 (inhibited root elongation)</td>
<td>S</td>
<td>500</td>
<td>g/kg</td>
<td>&lt;50</td>
<td>50–500</td>
<td>500</td>
</tr>
<tr>
<td>Soil respiration test</td>
<td>336-hr LC50</td>
<td>L</td>
<td>100</td>
<td>%</td>
<td>&lt;50</td>
<td>20–75</td>
<td>75–100</td>
</tr>
<tr>
<td></td>
<td>336-hr EC50</td>
<td>S</td>
<td>500</td>
<td>g/kg</td>
<td>&lt;50</td>
<td>50–500</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>100</td>
<td>%</td>
<td>&lt;50</td>
<td>20–75</td>
<td>75–100</td>
</tr>
</tbody>
</table>

a S = solid, L = aqueous liquid, includes water samples and elutriate or leachate. Nonaqueous liquids are evaluated on an individual basis because of variations in samples, such as vehicle, percent organic vehicle, and percent solids.

b MAD = Maximum applicable dose.
c LC50 = Calculated concentration expected to kill 50% of population within the specified time interval. EC50 = Calculated concentration expected to produce effect in 50% of population within the specified time interval.

Table 6.56 Useful Species and Life Stages for Aqueous Phase Testing

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
</tr>
<tr>
<td>Cold Water</td>
<td></td>
</tr>
<tr>
<td>Brook trout</td>
<td>Salvelinus fontinalis</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>Oncorhynchus kisutch</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Salmo gairdneri</td>
</tr>
<tr>
<td>Warm Water</td>
<td></td>
</tr>
<tr>
<td>Bluegill</td>
<td>Lepomis macrochirus</td>
</tr>
<tr>
<td>Channel catfish</td>
<td>Ictalurus punctatus</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>Pimephales promelas</td>
</tr>
<tr>
<td><strong>Benthic Invertebrates</strong></td>
<td></td>
</tr>
<tr>
<td>Cold Water</td>
<td></td>
</tr>
<tr>
<td>Stoneflies</td>
<td>Pteronarcys spp.</td>
</tr>
<tr>
<td>Crayfish</td>
<td>Pacifastacus leniusculus</td>
</tr>
<tr>
<td>Mayflies</td>
<td>Baetis spp. or Ephemerella spp.</td>
</tr>
<tr>
<td>Warm Water</td>
<td></td>
</tr>
<tr>
<td>Amphipods</td>
<td>Hyalella azteca</td>
</tr>
<tr>
<td>Cladocera</td>
<td>Daphnia magna or D. pulex, Ceriodaphnia spp.</td>
</tr>
<tr>
<td>Crayfish</td>
<td>Orconectes spp., Cambarus spp., Procambarus spp.</td>
</tr>
<tr>
<td>Mayflies</td>
<td>Hexagenia limbata or H. bilineata</td>
</tr>
<tr>
<td>Midge</td>
<td>Chironomus tentans or C. riparius</td>
</tr>
<tr>
<td><strong>Algae</strong></td>
<td></td>
</tr>
<tr>
<td>Green algae</td>
<td>Selenastrum capricornutum</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Microtox</td>
<td>Photobacterium phosphoreum</td>
</tr>
</tbody>
</table>


Microtox, then the assumption may be made that Microtox responses are related (noting statistical confidence) to the other surrogate responses. This will allow for the analysis of many more samples, because Microtox requires a few hours rather than days to complete, and many samples can conveniently be evaluated at one time.

When conducting ecotoxicity evaluations, it is important that one understand what effects sample collection, processing manipulation, and exposure design have on the observed toxicity response. Is this response similar to what is occurring in the field or is it simply an artifact of the method? A thorough discussion of this critical issue is beyond the scope of this book. See ASTM (1991) and Burton (1991) for additional information. For sediment testing, these effects are particularly significant, as sample integrity is easily disrupted, altering bioavailability and partitioning of toxicants. Sediment test phases include whole sediments, interstitial water, elutriate, or other extractable phases. Each has associated strengths and weaknesses (Table 6.57) (Burton 1991).
### Table 6.57 Sediment Phases Used in Toxicity Tests

<table>
<thead>
<tr>
<th>Phase</th>
<th>Strengths</th>
<th>Weaknesses</th>
<th>Routine Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable phase (XP)</td>
<td>• Use with all sediment types</td>
<td>• Ecosystem realism: Bioavailability unknown, chemical alteration</td>
<td>• Rapid screen</td>
</tr>
<tr>
<td></td>
<td>• Sequentially extract different degrees of bioavailable fractions</td>
<td></td>
<td>• Unique endpoints component of test battery</td>
</tr>
<tr>
<td></td>
<td>• Greater variety of available assay endpoints</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Determine dose response</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Use with all sediment types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elutriate phase (EP)</td>
<td>• Readily available fraction</td>
<td>• Ecosystem realism: Only one oxidizing condition used; only one solid: water ratio; exposure for extended period of one-phase condition that never occurs in situ or never occurs in equilibrium in situ</td>
<td>• Rapid screen</td>
</tr>
<tr>
<td></td>
<td>• Mimics anoxic toxic environmental process</td>
<td></td>
<td>• Endpoints not possible with WS</td>
</tr>
<tr>
<td></td>
<td>• Large variety of available assay endpoints</td>
<td></td>
<td>• Dredging evaluations</td>
</tr>
<tr>
<td></td>
<td>• Methods relatively standardized</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Determine dose response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial water (IW)</td>
<td>• Direct route of uptake for some species</td>
<td>• Extract conditions vary with investigator</td>
<td>• Rapid screen</td>
</tr>
<tr>
<td></td>
<td>• Semi-direct exposure phase for some species</td>
<td>• Filtration affects response, sometimes used</td>
<td>• Endpoints not possible with WS</td>
</tr>
<tr>
<td></td>
<td>• Large variety of available assay endpoints</td>
<td>• Cannot collect IW from some sediments</td>
<td>• Initial surveys</td>
</tr>
<tr>
<td></td>
<td>• Methods of exposure relatively standardized</td>
<td>• Limited volumes can be collected efficiently</td>
<td>• Sediment criteria</td>
</tr>
<tr>
<td></td>
<td>• Determine dose response</td>
<td>• Optimal collection method unknown, constituents altered by all methods</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Sediment quality criteria</td>
<td>• Exposure phase altered chemically and physically when isolated from WS</td>
<td></td>
</tr>
<tr>
<td>Whole sediment (WS)</td>
<td>• Use with all sediment</td>
<td>• Flux between overlying water and sediment unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Relative realism high</td>
<td>• Relationship to and between some organisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Determine dose response</td>
<td>• uncertainty: burrowers, epibenthic, water column species, filter feeders, selective filtering, life cycle vs. pore water exposure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Holistic (whole) versus reductionist toxicity approach (water, IW, EP, and XP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Use site or reconstituted water to isolate WS</td>
<td>• Methods relatively standardized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Real measure integrating all key components, eliminating extraneous influences</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Sediment quality criteria may be determined</td>
<td>• Some physical/chemical/microbiological alteration from field collection</td>
<td>• Rapid screen</td>
</tr>
<tr>
<td></td>
<td>• Resuspension/suspended solids effects assessed.</td>
<td>• Dose–response methods tentative</td>
<td>• Chronic studies</td>
</tr>
<tr>
<td>In situ* (NS)</td>
<td></td>
<td>• Testing more difficult with some species and some sediments</td>
<td>• Initial surveys</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Few standard methods</td>
<td>• Sediment criteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Indigenous biota may be present in sample</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Fee methods and endpoints</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Not as rapid as some assay systems</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mesocosms variable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Predation by indigenous biota</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Resuspension effects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Intensive system monitoring</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sediment criteria</td>
<td></td>
</tr>
</tbody>
</table>

* Organism exposed in situ in natural systems, pond/stream mesocosms, or lake limnocorrals.

Table 6.58 Optimal Toxicity Assay Considerations

1. Verification components
   - Ecosystem relevance
   - Species sensitivity patterns
   - Appropriate test phase
   - Short or long exposure period
   - Definitive response dynamics

2. Resource components
   - Organism availability
   - Laboratory availability
   - Expertise required
   - Expense and time required

3. Standardization components
   - Approved standard methods
   - Reference database
   - Interlaboratory validation
   - Quality assurance and quality control criteria


Case Study: Example Use of Microtox to Identify Sources and Controllability of Stormwater Toxicants

A series of projects were sponsored by the EPA to investigate sources and treatability of toxicants in stormwater (Pitt et al. 1995, 1999). The first project phase investigated typical toxicant concentrations in stormwater, the origins of these toxicants, and storm and land-use factors that influenced these toxicant concentrations. The second project phase investigated the control of stormwater toxicants using a variety of conventional bench-scale treatment processes. The Microtox 100% sample toxicity screening test by Azur Environmental (was Microbics, Inc.) was selected for this research because of its unique capabilities: it is a rapid procedure (requiring about 1 hour) and only requires small (<40 mL) sample quantities. The Microtox toxicity test uses marine bioluminescence bacteria and monitors the light output for different sample concentrations. About 1 million bacteria organisms are used per sample, resulting in highly repeatable results. The more toxic samples produce greater stress on the bacteria test organisms, which results in a greater light attenuation compared to the control sample. It must be stressed that the Microtox toxicity screening test was not used to indicate the absolute toxicities of the samples nor to predict the toxic effects of the stormwater runoff on receiving waters during this research. It was used as a control parameter to indicate relative toxicities of different source flows and to measure relative benefits of different control options. The precision and bias of the Microtox test were easy to measure and control during these tests, which also strongly favored its use for our purposes. The following paragraphs describe the results of these tests and indicate the types of information that can be obtained using a toxicity screening procedure, such as the Microtox test.

Phase 1 — Sources of Stormwater Toxicants

The first project phase included the collection and analysis of 87 urban stormwater runoff samples from a variety of source areas under different rain conditions. All of the samples were analyzed in filtered (0.45-µm filter) and nonfiltered forms to enable partitioning of the toxicants into particulate and filterable forms. The samples were all obtained from the Birmingham, AL, area. Samples were obtained from shallow flows originating from homogeneous sources. These
data were used to evaluate the effects of different land uses and source areas, plus the effects of rain characteristics, on sample toxicant concentrations and toxicity. Organic pollutants were analyzed using two gas chromatographs, one with a mass selective detector (GC/MSD) and another with an electron capture detector (GC/ECD). The pesticides were analyzed according to EPA method 505, while the base neutral compounds were analyzed according to EPA method 625 (but using only 100-mL samples). The pesticides were analyzed on a Perkin Elmer Sigma 300 GC/ECD using a J&W DB-1 capillary column (30 m by 0.32 mm ID with a 1-µm film thickness). The base neutrals were analyzed on a Hewlett Packard 5890 GC with a 5970 MSD using a Supelco DB-5 capillary column (30 m by 0.25 mm ID with a 0.2-µm film thickness).

Metallic toxicants were analyzed using a graphite furnace-equipped atomic absorption spectrophotometer (GFAA). EPA methods 202.2 (Al), 213.2 (Cd), 218.2 (Cr), 220.2 (Cu), 239.2 (Pb), 249.2 (Ni), and 289.2 (Zn) were followed in these analyses. A Perkin Elmer 3030B atomic absorption spectrophotometer was used after nitric acid digestion of the samples. Previous research (Pitt and McLean 1986; EPA 1983a) indicated that low detection limits were necessary in order to measure the filtered sample concentrations of the metals, which would not be achieved by use of a standard flame atomic absorption spectrophotometer. Low detection limits would enable partitioning of the metals between the solid and liquid phases to be investigated, an important factor in assessing the fates of the metals in receiving waters and in treatment processes.

**Comparison of Microtox with Other Toxicity Tests** — The Microtox procedure was compared with about 20 different laboratory bioassay tests using 20 stormwater and CSO samples. Conventional bioassay tests were conducted using freshwater organisms at the EPA’s Duluth, MN, laboratory and using marine organisms at the EPA’s Narragansett Bay, RI, laboratory. In addition, other toxicity tests were also conducted at the Environmental Health Sciences Laboratory at Wright State University, Dayton, OH. The comparison tests were all short-term tests. However, some of the tests were indicative of chronic toxicity (life cycle tests and the marine organism sexual reproduction tests, for example), whereas the others are classically considered as indicative of acute toxicity (Microtox and the fathead minnow tests, for example). The following list shows the major tests that were conducted by each participating laboratory:

- **University of Alabama at Birmingham, Environmental Engineering Laboratory**
  Microtox bacterial luminescence tests (10-, 20-, and 35-min exposures) using the marine *Photobacterium phosphoreum*

- **Wright State University, Biological Sciences Department**
  Macrofaunal toxicity tests:
  - *Daphnia magna* (water flea) survival
  - *Lemma minor* (duckweed) growth
  - *Selenastrum capricornutum* (green alga) growth
  Microbial activity tests (bacterial respiration):
  - Indigenous microbial electron transport activity
  - Indigenous microbial inhibition of β-galactosidase activity
  - Alkaline phosphatase for indigenous microbial activity
  - Inhibition of β-galactosidase for indigenous microbial activity
  - Bacterial surrogate assay using O-nitrophenol-β-D-galactopyranoside activity and *Escherichia coli*

- **EPA Environmental Research Laboratory, Duluth, MN**
  - *Ceriodaphnia dubia* (water flea) 48-hour survival
  - *Pimephales promelas* (fathead minnow) 96-hour survival

- **EPA Environmental Research Laboratory, Narraganset Bay, RI**
  - *Champia parvula* (marine red alga) sexual reproduction (formation of cystocarps after 5 to 7 days exposure)
  - *Arbacua punctulata* (sea urchin) fertilization by sperm cells
Therefore, the tests represented a range of organisms that included fish, invertebrates, plants, and microorganisms.

Table 6.59 summarizes the results of the toxicity tests. The *C. dubia*, *P. promelas*, and *C. parvula* tests experienced problems with the control samples, and those results are therefore uncertain. The *A. punctulata* tests on the stormwater samples also had a potential problem with the control samples. The CSO test results (excluding the fathead minnow tests) indicated that from 50 to 100% of the samples were toxic, with most tests identifying the same few samples as the most toxic. The toxicity tests for the stormwater samples indicated that 0 to 40% of the samples were toxic. The Microtox screening procedure gave rankings similar to those of the other toxicity tests.

All of the Birmingham samples represented separate stormwater. However, as part of the Microtox evaluation, several CSO samples from New York City were also tested to compare the different toxicity tests.

<table>
<thead>
<tr>
<th>Sample Series</th>
<th>Combined Sewer Overflows, %</th>
<th>Stormwater, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtox marine bacteria</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td><em>C. dubia</em></td>
<td>60</td>
<td>0*</td>
</tr>
<tr>
<td><em>P. promelas</em></td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td><em>C. parvula</em></td>
<td>100</td>
<td>0*</td>
</tr>
<tr>
<td><em>A. punctulata</em></td>
<td>100</td>
<td>0*</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td><em>L. minor</em></td>
<td>50*</td>
<td>0*</td>
</tr>
</tbody>
</table>

* Results uncertain, see text.

**Source Area Sampling Results** — Thirteen organic compounds, out of more than 35 targeted compounds analyzed, were detected in over 10% of all samples. The greatest detection frequencies were for 1,3-dichlorobenzene and fluoranthene, which were each detected in 23% of the samples. The organics most frequently found in these source area samples (i.e., PAHs, especially fluoranthene and pyrene) were similar to the organics most frequently detected at outfalls in prior studies (EPA 1983a). Roof runoff, parking area, and vehicle service area samples had the greatest detection frequencies for the organic toxicants. Vehicle service areas and urban creeks had several of the observed maximum organic compound concentrations. Most of the organics were associated with the nonfiltered sample portions, indicating an association with the particulate sample fractions. The compound 1,3-dichlorobenzene was an exception, having a significant dissolved fraction.

In contrast to the organics, the heavy metals analyzed were detected in almost all samples, including the filtered sample portions. The nonfiltered samples generally had much higher concentrations, with the exception of zinc, which was associated mostly with the dissolved sample portion (i.e., not associated with the suspended solids). Roof runoff generally had the highest concentrations of zinc, probably from galvanized roof drainage components, as previously reported by Bannerman et al. (1983). Parking and storage areas had the highest nickel concentrations, while vehicle service areas and street runoff had the highest concentrations of cadmium and lead.

Replicate samples were collected from several source areas at three land uses during four different storm events to statistically examine toxicity and pollutant concentration differences due to storm and site conditions. These data indicated that variations in Microtox toxicities and organic toxicant concentrations may be better explained by rain characteristics than by differences in sampling locations. As an example, high concentrations of many of the PAHs were more likely associated with long antecedent dry periods and large rains, than by any other storm or sampling location parameter.
Phase 2 — Laboratory-Scale Toxicant Reduction Tests

The Phase 2 tests examined toxicant treatability for a variety of conventional bench-scale treatment processes. The data from Phase 1 identified the critical source areas (storage/parking and vehicle service areas, which generally had the highest toxicant concentrations) for study during the second research phase.

The objective of the second research phase was to obtain relative measurements of sample toxicity improvements for different stages of each bench-scale treatment method to indicate the relative effectiveness of different treatment efforts and processes. To meet this objective and considering resource restraints on cost and time, the Microtox screening toxicity test was chosen to indicate relative changes in toxicity.

The selected source area runoff samples all had elevated toxicant concentrations compared to other urban source areas, allowing a wide range of laboratory partitioning and treatability analyses to be conducted. The treatability tests conducted were:

1. Settling column (37 mm × 0.8 m Teflon column)
2. Flotation (series of eight glass, narrow-neck, 100-mL volumetric flasks)
3. Screening and filtering (series of 11 stainless steel sieves, from 20 to 106 µm, and a 0.45-µm membrane filter).
4. Photodegradation (2-L glass beaker with a 60-watt, broad-band, incandescent light placed 25 cm above the water, stirred with a magnetic stirrer with water temperature and evaporation rate also monitored)
5. Aeration (the same beaker arrangement as above, without the light, but with filtered compressed air keeping the test solution supersaturated and well mixed)
6. Photodegradation and aeration combined (the same beaker arrangement as above, with compressed air, light, and stirrer)
7. Undisturbed control sample (a sealed and covered glass jar at room temperature)

Each test (except for filtration, which was an “instantaneous” test) was conducted over a duration of 3 days. Plots of the toxicity reductions observed during each treatment procedure examined, including the control measurements, were prepared. The plots were grouped according to source area sampling location and the treatment type. Figures 6.145 through 6.147 are plots of toxicity reductions associated with filtering selected samples through different sized screens. Significant and important toxicity reductions are associated with screening using the smaller apertures.

The highest toxicant reductions were obtained by settling for at least 24 hours (providing at least 50% reductions for all but two samples), screening through at least a 40-µm screen (20 to 70% reductions), and aeration and/or photodegradation for at least 24 hours (up to 80% reductions). Increased settling, aeration or photodegradation times, and screening through finer meshes, all reduced sample toxicities further. The flotation tests produced floating sample layers that generally increased in toxicity with time and lower sample layers that generally decreased in toxicity with time, as expected; however, the benefits were quite small (less than 30% reduction).

These tests indicate the wide-ranging behavior of these related samples for the different treatment tests. Some samples responded poorly to some tests, while other samples responded well to all of the treatment tests. Any practical application of these treatment unit processes would therefore require a treatment train approach, subjecting critical source area runoff to a combination of processes in order to obtain relatively consistent overall toxicant removal benefits.

Phase 3 – Pilot-Scale Demonstration of the Multichambered Treatment Train (MCTT)

The last research phase included a pilot-scale test of the most promising treatment processes suitable for small critical source areas. This device consists of a series of chambers, including an initial grit and aeration chamber, an intermediate tube settler with oil sorbents, and a final mixed
sand/peat filter. Extensive testing of PAHs, phthalate esters, phenols, pesticides, metals, toxicity screening, chemical oxygen demand, pH, conductivity, turbidity, hardness, sodium adsorption ratio, major ions, particle sizes, solids, and nutrients was performed on filtered and unfiltered samples during 12 rains at the inlets and outlets of each component of the treatment train. The results from this pilot-scale test were confirmed by full-scale installations in Wisconsin constructed and monitored by the WI DNR. The MCTT units have been shown to be extremely effective, with >90%
removal of heavy metals and most organic toxicants. Caltrans (California Department of Transportation) is currently constructing and monitoring three MCTT units for treatment of runoff from a maintenance area and from parking lots in Los Angeles.

This research showed the usefulness of a toxicity screening test in evaluating sources of stormwater toxicants and in developing and testing control technologies. It would have been prohibitively expensive to base this research solely on chemical analyses of specific metallic and organic toxicants, although toxicants were specifically monitored as part of the demonstration projects to show applicability of results.

**Standard Testing Protocols: Sediments**

The release of the EPA Contaminated Sediment Management Strategy and Sediment Quality Inventory compiled the limited sediment data (only 4% of monitored sites had toxicity data) and documented that adverse effects are probable from sediments at 26% (>5000) of sites surveyed (EPA 1998). A recent random survey of sediments in North Carolina’s estuaries found from 19 to 36% had contaminant levels known to cause toxicity and 13% had few to no living organisms (Pelly 1999). These areas are dominated by agricultural watershed inputs. The paucity of sediment toxicity information and the focus of past sediment surveys on industrialized waterways raises the question of whether the extent of sediment contamination is actually much greater than envisioned. Since chemicals, nutrients, and pathogens readily sorb to sediments, sediment contamination is likely in depositional areas of urban and agricultural watersheds (Burton 1992a,b; Burton et al. 1987). Contaminated sediments have been shown to severely impact aquatic ecosystems (e.g., Burton 1992a,b; EPA 1998) and are the source of fish contamination and advisories in many parts of the nation (EPA 1998). For this reason, it is essential that their contribution to use impairment be determined.

By the mid-1990s, standardized methods for whole-sediment toxicity testing occurred within the EPA, American Society for Testing and Materials (ASTM), and Environment Canada. These tests measured acute (short-term ≤ 10 days) toxicity in benthic macroinvertebrates such as the amphipods *Hyalella azteca*, *Rhepoxynius abronius*, *Ampelisca abdita*, *Eohaustorius estuarius*, and *Leptocheirus plumulosus*, and the midges *Chironomus tentans* and *Chironomus riparius* (EPA 2000c). The primary response measured was mortality, but in the case of the midge, growth was included and reburial was an additional endpoint for the *Rhepoxynius abronius*. These whole-sediment tests have been useful at testing sediment contamination (Figure 6.148) and provide information on chemical bioavailability. A large number of other species have been used for determining the toxicity of sediments, ranging from bacteria to fish and amphibians (Burton 1991). Comparisons of their sensitivities have shown a wide range of responses to different types of sediment contamination, with an equally wide range of discriminatory power (ability to detect differences between samples) (Burton et al. 1996a). This reality suggests that more than one or two species may be necessary to determine with certainty whether or not sediment contamination is ecologically significant (EPA 1994c).

Unfortunately, most of the test methods are focused on acute and not chronic toxicity. The measures of acute toxicity are often not adequate to detect the impacts on benthic communities. For instance, the 10-day test with *Rhepoxynius abronius* was not sensitive enough to describe the loss of amphipods from the Lauritzen Channel in San Francisco Bay (Swartz et al. 1994). In reality, chronic toxicity is the more pervasive problem, and it is the chronic responses, such as changes in reproduction, that lead to population level responses. Late in 1999, the EPA released its first standardized methods for determining chronic toxicity, specifically focused on growth and reproduction in *Hyalella azteca* and *Chironomus tentans* (as described in Benoit et al. 1997; Ingersoll et al. 1998). While these methods greatly aid our ability to determine if sediments are chronically toxic, their long duration and increased costs may impede their widespread adoption by state agencies.
Beyond the standard tests, there have been a large number of tests with a wide range of marine benthos that may lead to better, or at least more effective, measures of chronic toxic response. For example, tests with marine amphipods have already been described in the literature to optimize the conditions for a 28-day test to examine growth and reproduction with *Leptochirius plumulosus* (Gray et al. 1998). Additional tests make use of organisms with shorter life spans, such as marine copepods, and can sort out differential response to different life stages (Green et al. 1996). These copepods are also useful in more community structure-based assessments, such as in the use of microcosms (Chandler et al. 1997). These meiobenthos may well be useful for developing standardized chronic tests since life cycle tests can be completed in 15 to 25 days and the organisms have been found to be sensitive to sediment-associated toxicants under laboratory and field conditions (Coull and Chandler 1992). Tests with organisms having shorter life spans and methods that include mixed assemblages in microcosms linked with single species tests provide insight into the functioning of communities. These new methods will help bridge the gap between our field observations and the cause–effect links that can be established in the laboratory.

There are several reasons the “water column” species used in WET tests are useful for assessments of sediments. Aquatic organisms rarely exclusively inhabit one media during their life cycle. Many “pelagic” organisms may graze on surficial sediments and even encounter pore waters. For example, the often-used “water column” surrogate, the fathead minnow (*Pimephales promelas*) is an omnivore, ingesting a mixture of detritus and invertebrates (Lemke and Bowan 1998) and frequently feeding on sediment surfaces. The zooplankton, *Daphnia magna*, grazes on surficial sediments in whole-sediment toxicity assays. The responses of WET tests have been highly predictive of indigenous benthic community responses at many sites (Dickson et al. 1996; Eagleson et al. 1990). Many vertebrate and invertebrate species have some link to sediments and have been shown to be adversely affected by sediment contamination through toxicity and effects of bioaccumulation (e.g., Baumann and Harshbarger 1995; Benson and Di Giulio 1992; Burgess and Scott 1992; Burton 1989, 1991, 1992a,b, 1999; Burton and Scott 1992; Burton and Stemmer 1988; Burton et al. 1989, 1996a,b,c; Chapman et al. 1992; Lamberson et al. 1992; Lester and McIntosh 1994; Ludwig et al. 1993; Mac and Schmitt 1992; Maruya and Lee 1998).

For most stormwater effect evaluations, sediment toxicity determinations should focus on sampling surficial sediments (approximately to 2 cm) during low flow conditions and use whole-sediment exposures. During high flow conditions, suspended-sediment assays can be conducted in
the laboratory or in situ. The EPA and ASTM has developed standard guides for whole-sediment toxicity and bioaccumulation testing using invertebrates (ASTM 2000; EPA 2000c). Specific species guidance exists for *H. azteca*, *C. tentans*, and *C. riparius* (Figures 6.149 through 6.151). ASTM methods are also available for *Daphnia* and *Ceriodaphnia* spp. and resuspension testing. For additional test method references, see Burton (1991). Appendix D includes summaries of toxicity test methods for aqueous samples, using fish, cladocerans, algae, benthic invertebrates, and Microtox, which may be modified for sediment testing (Burton 1991). Testing suspended-sediment toxicity in the laboratory presents a logistical challenge. It is difficult to maintain a constant suspended solids concentration yet keep flow velocity and mixing turbulence reduced so as not to overly stress the test species, such as *Daphnia* sp. or *P. promelas* larvae. Relatively simple recirculation systems have been described by Hall (1986), Schuytema et al. (1984), and Schrap and Opperhuizen (1990). A preferred method of testing suspended solids is either with on-site mobile laboratories (using a flow-through pump system) or with in situ exposure chambers (Sasson and Burton 1991; Ireland et al. 1996; Burton and Moore 1999).

Standardized test methods have been developed for chronic toxicity testing of freshwater sediments. The EPA and ASTM have nearly identical methods (EPA 2000c; ASTM 2000). These methods are for *H. azteca* and *C. tentans* and extend for 42 to 60 days.

*Hyalella azteca* are routinely used to assess the toxicity of chemicals in sediments (e.g., Burton et al. 1989, 1996c; Burton 1991). Test duration and endpoints recommended in previously developed standard methods for sediment testing with *H. azteca* include 10-day survival and 10- to 28-d survival and growth. Short-term exposures, which only measure effects on survival, can be used to identify high levels of contamination, but may not be able to identify moderately contaminated sediments.

This method can be used to evaluate potential effects of contaminated sediment on survival, growth, and reproduction of *H. azteca* in a 42-day test. The sediment exposure starts with 6- to 8-day-old amphipods. On Day 28, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Typically, amphipods are first in amplexus at about Day 21 to 28 with release of the first brood between Day 28 to 42. Endpoints measured include survival (Day 28, 35, and 42), growth (dry weight measured on Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). The EPA and ASTM state that a subset of endpoints may be measured with minor method modifications.

Reproduction in amphipods is measured by exposing them in sediment until a few days before the release of the first brood. The amphipods are then sieved from the sediment and held in water to determine the number of young produced. This test design allows a quantitative measure of reproduction. One limitation to this design is that amphipods might recover from effects of sediment exposure during this holding period in clean water; however, amphipods are exposed to sediment during critical developmental stages before release of the first brood in clean water.

**Figure 6.149** EPA whole sediment, overlying water renewal design. **Figure 6.150** The amphipod *Hyalella azteca*, also known as the scud.
The midge *Chironomus tentans* has been used extensively in the short-term assessment of chemicals in sediments (e.g., Burton 1991; Burton et al. 1996c), and standard methods have been developed for testing with this midge using 10-day exposures (EPA 2000c). *Chironomus tentans* is a good candidate for long-term toxicity testing because it normally completes its life cycle in a relatively short period of time (25 to 30 days at 23°C), and a variety of developmental (growth, survivorship) and reproductive (fecundity) endpoints can be monitored. In addition, emergent adults can be readily collected, so it is possible to transfer organisms from the sediment test system to clean, overlying water for direct quantification of reproductive success. In Europe and Canada, the chronic midge method ends after emergence.

The long-term sediment toxicity test with the midge, *Chironomus tentans*, is a life-cycle test in which the effects of sediment exposure on survival, growth, and emergence are measured. In addition, reproduction endpoints may be assessed. Survival is determined at 20 days and at the end of the test (about 50 to 65 days). Growth is determined at 20 days, which corresponds to the 10-day endpoint in the 10-day *C. tentans* growth test started with 10-day-old larvae. From Day 23 to the end of the test, emergence is monitored daily. Each treatment of the life-cycle test is ended separately when no additional emergence has been recorded for 7 consecutive days (the 7-day criterion). When no emergence is recorded from a treatment, ending of that treatment should be based on the control sediment using this 7-day criterion. EPA and ASTM state that minor modifications to the basic methods and a subset of endpoints may be used.

**In Situ Toxicity Testing**

An effective and accurate way to determine stormwater effects is through *in situ* toxicity testing. This may be done by placement of either artificial substrates (e.g., Hester–Dendy [OEPA 1989], rock- or mesh-filled baskets [EPA 1990b], foam [Henebry and Ross 1989], glass slides [APHA 1985]), side-stream chambers, or placing chambers-cages containing the test species into the stream or lake. The substrates or chambers must be secured to the stream bottom and be able to withstand high flow conditions. Some form of protective barrier might be necessary which might complicate flow-related effects on colonization.

*In situ* assessments of toxicity using confined organisms, while not new, have not been used traditionally in contaminant assessments (Burton et al. 1996b). A limited number of *in situ* exposures have been conducted to assess water or effluent toxicity. These assays have utilized adult fish, phytoplankton, amphipods, oligochaetes, and protozoans. Recent studies have shown the usefulness of *in situ* toxicity testing (Burton et al. 1996b; Chappie and Burton 1997; Crane et al. 1995; Monson et al. 1995; Sasson-Brickson and Burton 1991; Ireland et al. 1996; Bascombe et al. 1990; Ellis et al. 1995; Malby et al. 1995; Sarda and Burton 1995; Schulz 1996; Nichols et al. 1999; Pereira et al. 1999; Malby et al. 2000; Schulz and Liess 1999; Sibley et al. 1999). Determining the significance of sediment-associated contaminants requires an assessment of overlying water toxicity as organisms are exposed to both. This water-column exposure includes low and high flow conditions, in which water quality can vary markedly (Figure 6.152). Laboratory testing of wet-weather runoff samples has shown acute and chronic toxicity to a variety of species (e.g., Portele et al. 1982; Medeiros and Coler 1982; Medeiros et al. 1984; Ireland et al. 1996; Tucker and Burton 1999; Bailey et al. 2000). However, it is difficult to extrapolate results of these constant exposures with actual time-scale events.
in the field (Burton et al. 1996b; Tucker and Burton 1999; Burton and Moore 1999). Other in situ studies which have been used successfully in runoff studies include exposure of fish eggs (Pitt and Bissonnette 1984), artificial substrates for benthic invertebrate colonization and protozoa (e.g., Sayre et al. 1986), and use of transplants (Cherry 1996; Malley 1996).

There are several advantages to in situ testing. This approach removes sampling and laboratory-related errors from the assessment process, negating laboratory-to-field extrapolation uncertainties. Field conditions which may affect organism response and toxicity (and which are difficult to simulate in the laboratory) include sunlight, diurnal effects of temperature and oxygen, suspended solids, stressor(s) magnitude, frequency and duration, sediment integrity, spatial and temporal variation of physicochemical constituents, resident meio–microfaunal interactions, and other unknowns. Significant differences have been observed between laboratory and field testing. For example, acute toxicity to *C. dubia* in 48-hour exposures (Figure 6.153) was increased and overlying water reduced in the laboratory as compared to simultaneous in situ exposures (Figures 6.154 and 6.155) (Sasson-Brickson and Burton 1991). Ellis et al. (1992) observed acute and chronic toxicity to the amphipod, *Gammarus* sp., following storm event exposures in an urban stream. Death occurred up to 3 weeks following the storm and was related to elevated zinc concentrations in high-flow waters. Effects could also be correlated with *Gammarus* tissue levels of Zn. Kocan and Landolt (1990) exposed herring embryos both in the laboratory and in situ by placing 20 to 25 eggs on five glass slides, covering the slide holder with mesh and placing in situ. This system was not tested in fresh waters or in flowing waters.

Artifacts associated with sampling and manipulation (e.g., sieving and mixing of sediments) of the test samples are reduced in in situ assays. Such manipulations may disrupt sediment vertical contaminant gradients, thereby altering the contaminant exposure regime that organisms face in
the field (Sasson-Brickson and Burton 1991). *In situ* collection of interstitial water by deploying “peeper” devices has shown chemistry differences when compared to traditional collection methods using grab or core sampling (e.g., Adams 1991; Sarda and Burton 1995) and also when used for organism exposures (Fisher 1992; Figure 6.156).

*In situ* toxicity tests are more realistic than laboratory tests at integrating stressors (both measured and unmeasured), and can be used to study a variety of effects, such as photoinduced toxicity of PAHs (interactions with sunlight, solids, and contaminants), stormwater runoff (interactions of contaminants, suspended and dissolved solids, flow, and food), sediment-associated stressors, point source effluents, and contaminant gradients (Sasson-Brickson and Burton 1991; Ireland et al. 1996; Jones et al. 1995; Absil et al. 1996; Postma et al. 1994; Dickson et al. 1992; Roper and Hickey 1995; Hickey et al. 1995). Worms, bivalves, and fish have all been used *in situ* in bioaccumulation studies (e.g., Monson et al. 1995; Warren et al. 1995) with a need for linking critical body burdens to biological responses (Borgman 1996). Multiple stressors in the field usually occur in nonlinear, nonorthogonal combinations, challenging

**Figure 6.154** Sediment exposure chamber units secured in stream bed. (Reprinted with permission from Sasson-Brickson, G. and Burton, G.A., Jr. *In situ* and laboratory toxicity testing with *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.*, 10: 201–207, 1991. © SETAC, Pensacola, FL, U.S.A.)

**Figure 6.155** *Ceriodaphnia dubia* survival in laboratory (static and flow-through) whole sediment and site water (W) exposures; as compared to *in situ* exposures (whole sediment and overlying water, and overlying water (W) only).
biological systems in ways that are difficult at best to reproduce in the laboratory. So methods for teasing out the relative contributions of each stressor are often best conducted using a combination of in situ and laboratory-based experiments.

The integration of time-varying stressors (such as those related to wet-weather flow, pesticide runoff, or tidal inundation) is best conducted with field-deployed tests allowing continual exposure, as opposed to the grab sampling, static-type exposures of the laboratory. The first-flush of stormwater or pesticide runoff produces acute to sublethal responses to organisms exposed in situ (e.g., Herricks et al. 1994; Maltby et al. 1995; Crane et al. 1995; Waller et al. 1995b). Bivalve gape monitoring appears to be useful as an early warning indicator of effluent or stormwater toxicity (Waller et al. 1995a).

In situ methodologies can be extended to examine toxicological responses at the community level, for which they are much more cost effective than mesocosm studies (i.e., the laboratory analog). Typically, these experiments have been carried out by placing dosed sediments into the field (Berge 1990; Watzin et al. 1994) or by carrying out contaminant dosing in situ (Pridmore et al. 1991; Morrisey et al. 1996).

At the same time, the limitations of in situ toxicity tests should be recognized. Laboratory tests control variability of nontreatment factors much better than their in situ analogs. Deployment of caged organisms introduces the possibility of acclimation and transportation stress. If this is not monitored, data interpretation could be flawed. The in situ tests incorporate spatial and temporal variation, so the appropriate sampling design and analytical methods must be adapted to ensure there is adequate sensitivity and discriminatory power. The ease and practicality of in situ testing is site dependent. Deployment in intertidal or shallow water systems is easier than in deeper waters. Shallow subtidal deployment has the advantages of its inaccessibility to the public and reduced disturbance of sediment, especially in the case of very soft muds where trampling of intertidal sites can be a major problem. However, subtidal studies may be impacted by fishing trawls (e.g., Morrisey et al. 1996). In some areas, destruction of cages by vandals is problematic.

Primary considerations in the design and analysis of in situ testing approaches are the availability of food and potential starvation associated with exposures. The bioaccumulation and toxicity of contaminants is strongly influenced by food or feeding (Absil et al. 1996; Postma et al. 1994). Laboratory feeding often cannot duplicate either the quality or quantity of food present in the field. Stimulatory or inhibitory effects in these situations will likely be most marked for filter- or deposit-feeding organisms (Roper and Hickey 1995; Hickey et al. 1995).

Stressor exposures may be altered due to caging effects. Primary among these would be reduced flow, altered suspended solids or food, and interactions with predators, communities, or the food web. Depending on the organisms and the flow dynamics, cage design restricts flow to varying...
degrees associated with flow-through screens (Nowell and Jumars 1984). It is essential in stormwater evaluations to reduce flow velocity to protect cages and organisms. This, however, increases the uncertainty concerning flow-related interactions in the receiving water (Vogel 1994). Predator–prey effects, suspended solids concentration, and settling within the cage may be increased or reduced depending on the mesh size. Artifacts associated with in situ experiments are further discussed by DeWitt et al. (1996).

Other important issues with in situ toxicity testing are the controls and reference sites. Selection of the appropriate controls and references is partially dictated by the questions being addressed in the study. In order to ascertain where stressors exist, site controls may be needed as well as reference sites. A priori impressions of what constitutes a "good" reference site may be incorrect. Multiple reference sites may be desirable to adequately interpret the impact data and accommodate unexpected loss of in situ devices. Artificial (formulated) sediments are also useful tools for investigating effects of food and bioavailability controls in conjunction with in situ deployments.

In situ testing provides unique information that may not be provided by laboratory testing or community surveys. The laboratory environment is superior for mechanistic and single-stressor effect delineation. However, complex exposure dynamics and stressor interactions are difficult or impossible to reproduce in the laboratory and may best be studied in situ. Significant advancements in understanding ecotoxicological processes and in conducting site assessments will come from the creative use of laboratory and in situ testing, and community survey approaches. When properly used in an integrated weight-of-evidence approach, in situ testing should help reduce the uncertainties associated with evaluating contaminant and natural stressor effects in complex ecosystems.

Bioaccumulation

Why Evaluate Bioaccumulation?

Aquatic organisms are exposed to chemicals through their contact with water and sediment and ingestion of food. Many inorganic and organic chemicals have been found to accumulate in organisms. These chemicals may accumulate to levels that cause chronic toxicity or even death. One of the most common sources of tissue contamination is sediment-associated contaminants. This contamination has been linked via food web transfer to impacts on upper trophic levels. Such transfer occurs with mercury and some organochlorines, such as PCBs and DDT, that are not well biotransformed and are hydrophobic; however, with other chemicals, these connections are more difficult to establish. Some organics such as PAHs are metabolized by many organisms, so detection in tissues may indicate recent exposures. Metals are difficult to evaluate in tissues since many are essential and can be regulated by organisms. Bioconcentration factors cannot be used with metals (with the exception of methyl mercury) because they can be high or low depending on the organism, their surrounding media, the metals, and their adaptation — most of which are not clearly defined in a study. From modeling exercises, food web transfer of persistent contaminants is important for maintaining the chemical concentrations observed in upper trophic levels, and the benthic component is essential in accounting for the observed concentrations (Thomann et al. 1992; Morrison et al. 1996; see Chapter 8). Further, trophic transfer of sediment-associated contaminants has been documented in both freshwater systems (e.g., Lester and McIntosh 1994) and marine systems (e.g., Maruya and Lee 1998). This food web transfer does not have to be limited to the aquatic environment and connections have been made to terrestrial species, particularly birds (Froese et al. 1998). In Saginaw Bay, Lake Huron, tree swallows were found to accumulate PCBs from sediments. In some areas of the Great Lakes and in the Hudson River, NY, system reproductive damage has been observed for this species directly linked to PCBs (Bishop et al. 1999; McCarty and Secord 1999).
**Determining Bioaccumulation**

A useful way to establish a link between beneficial use impairment and contamination is by showing that exposure to sediment or stormwater runoff contaminants results in tissue residue and adverse effects in organisms. Because many factors appear to alter the bioavailability of contaminants in sediments and stormwaters, approaches to establish links between the body-residue concentrations and effects in aquatic organisms provide the insight to better link the toxic response directly to contaminants. The concept is based on the understanding that it is the dose at the receptor that is responsible for the toxic response and that the receptor concentration is proportional to the contaminant concentration in the organism. This leads to development of a database of the concentrations of contaminants responsible for toxic responses in organisms (McCarty and Mackay 1993). Data have been amassed over the course of the past several years that allow the direct comparison of some residue levels with acute and chronic effects (McCarty and Mackay 1993; Jarvinen and Ankley 1999; www.wes.army.mil/el/ered). However, the database is very limited at this time, and there is still need to establish a weight-of-evidence approach for developing the link between the observed response and the presence of contaminants in sediments. Currently, there is only one standardized EPA test for sediment bioaccumulation. It is a 28-day test with the oligochaete *Lumbriculus variegatus* (Figure 6.157).

Bioaccumulation has often been assessed with *in situ* studies to determine site-specific effects. These studies have primarily used caged mussels (marine) or fish (EPA 1987; Mac et al. 1990). In one approach, adult fish (*P. promelas*) are placed in mesh cages (10 fish per compartment, 4 compartments per cage) and exposed for 10 days *in situ*. This may also be done with benthic invertebrates (e.g., mussels, amphipods, and oligochaetes (e.g., *Lumbriculus variegatus*), providing there is adequate biomass for chemical analyses. Caution should be exercised when formulating conclusions from these studies because the organisms are not exposed for extended periods, they may not be able to ingest foods and surficial sediments due to their mesh-cage barrier, and they may be stressed due to caging. These factors alter toxicokinetics. These weaknesses can be addressed by also collecting resident target species (Table 6.60) and analyzing tissues (EPA 2000a,b). Target species should be large adults that are upper trophic level (top predator) and/or bottom feeders, and they should be collected prior to winter yet well after spawning. Nonmigratory species are preferred, and their commercial or sport fishing importance should be considered. Samples should be processed as described in Appendix D. The decision of whether to analyze whole fish or select target organs (e.g., gills, liver, kidneys) depends on the study objective and concerns over food chain or human health effects.

Residue information should be interpreted with caution (as discussed above with metals); however, guidance exists for calculating fish consumption advisories (EPA 2000a). There is little information available on what constitutes a significant tissue concentration, and correlations with adverse effects are usually lacking. Many contaminants are present for days or less (e.g., synthetic pyrethroids), rapidly metabolized (e.g., synthetic pyrethroids, organophosphates), biotransformed (e.g., polycyclic aromatic hydrocarbons), or only present in the environment seasonally (e.g., herbicides, insecticides). The U.S. Food and Drug Administration and the U.S. Fish and Wildlife Service have some effect-level information for a few common contaminants. For further information, see EPA (1982, 2000a), Carlton and Klug (1990), and Mac and Schmidt (1992).
Table 6.60 Target Fish Species for Use in Tissue Analysis

I. Target Species (East of Appalachian Mountains)

***Brook trout (Salvelinus fontinalis) **Bluegill (Lepomis macrochirus)
***Small mouth bass (Micropterus dolomieui) **Pumpkinseed (Lepomis gibbosus)
***Large mouth bass (Micropterus salmoides) **Black crappie (Pomoxis nigromaculatus)
***Channel catfish (Ictalurus punctatus) **Striped bass (Morone saxatilis)
**Brown trout (Salmo trutta) *Carp (Cyprinus carpio)
**Rainbow trout (Salmo gairdnerii)

II. Target Species (West of Appalachian Mountains and East of Rocky Mountains)

***Rainbow trout (Salmo gairdnerii) **Yellow perch (Perca flavescens)
***Brook trout (Salvelinus fontinalis) **Walleye (Stizostedion vitreum)
***Small mouth bass (Micropterus dolomieui) **Bluegill (Lepomis macrochirus)
***Large mouth bass (Micropterus salmoides) **Brown trout (Salmo trutta)
***Channel catfish (Ictalurus punctatus) *Carp (Cyprinus carpio)
**Striped bass (Morone saxatilis)

III. Target Species (West of and including Rocky Mountains)

***Rainbow trout (Salmo gairdnerii) **Bluegill (Lepomis macrochirus)
***Brook trout (Salvelinus fontinalis) **Striped bass (Morone saxatilis)
***Small mouth bass (Micropterus dolomieui) *Cutthroat trout (Salmo clarki)
***Large mouth bass (Micropterus salmoides) *Brown trout (Salmo trutta)
***Channel catfish (Ictalurus punctatus) *Carp (Cyprinus carpio)

*** Preferred target species.
** Good target species.
* Acceptable target species.


Emerging Tools for Toxicity Testing

Semipermeable Membrane Devices (SPMDs)

While no standard methods exist, SPMDs have been reported widely in recent years as an excellent passive, in situ sampling device for organic contaminants in water and in air (Huckins et al. 1999; Axelman et al. 1999; Peterson et al. 1995; Sabaliunas et al. 1998; Petty et al. 1998; Woolgar and Jones 1999; Zabik et al. 1992; Prest et al. 1995, 1992). Granmo et al. (2000) recently conducted tests in marine waters in Sweden using SPMDs for comparison with bioaccumulation of organochlorine compounds (chlorobenzenes, chlorophenols, and PCBs) in feral and caged mussels and the concentrations found in sediment and the associated water column. Short-term exposures (30-day) of SPMDs and caged mussels were used to find whether the high pollutant concentrations found in the sediments were associated with recent or older industrial discharges. Feral mussels were also analyzed after longer exposure periods. They found that the test approach using the combination of SPMDs and mussels allowed the detection of short-term changes of discharges of these organochlorine compounds, especially considering that the SPMDs were found to be more effective at concentrating some of the target compounds.

The devices are generally polymeric (such as low-density polyethylene) tube bags containing a neutral lipid (such as triolein, iso-octane, 2,2,4-trimethylpentane). These bags are placed in receiving waters for a period of days and then recovered and the contents analyzed using gas chromatography/mass spectrometry, or high-pressure liquid chromatography for target compounds. The concentrations accumulated in the bags have been found to be relatively similar to what is
accumulated in resident fish and shellfish. However, the concentrations may be higher or lower by several-fold and vary in their relationship to each other. This method has the advantage of being easy to deploy and retrieve, and can sample compounds found at a specific site that are in the water column during a specified period of time (unlike fish, which migrate to different areas). In addition, biological organisms are not sacrificed for the analyses. Extended exposures may result in biofouling of the bag and care must be taken to ensure adequate field blanks are used to assess that no water-related contamination has occurred.

**DNA Fingerprinting**

Another novel assessment tool to measure stress is genetic markers (as discussed above). Randomly amplified polymorphic DNA (RAPD) markers have proven promising for determining differences in genetic variability in populations (Williams et al. 1990, 1991). Other studies (Sternberg et al. 1996; Ellis et al. 1997) showed highly significant differences in DNA profiles between benthic invertebrates from stressed and nonstressed sites. This inexpensive and quick assay shows the number and size of distinctive DNA profiles of genomic DNA from each organism. Because RAPD-PCR primers are not designed to amplify specific target sequences, the amplified loci are anonymous, scattered throughout the genome, and are not associated with stressor adaptation (neutral markers) (Williams et al. 1990). RAPD-PCR products are often highly polymorphic within naturally occurring populations and have proven to be excellent indicators of genetic diversity (Clark and Lanigan 1993).

**Biological Toxicity Fractionations**

After toxicity is identified in receiving waters, researchers commonly attempt to identify the toxicants responsible for the observed effects through toxicity identification evaluation (TIE) studies. Numerous TIE protocols have been used. Figure 6.158, from Lopes et al. (1995), is one example that was used in association with a stormwater toxicity study conducted in Phoenix, AZ. Acute toxicity of stormwater was found to occur, especially to fathead minnows, and was likely to degrade the quality of the receiving water (the Salt River).

This test protocol involved first conducting toxicity tests to identify stormwater that was toxic (>20% mortality after 24 hours). The toxic stormwater was then subjected to different extractions to selectively remove various pollutants from the stormwater, after which additional toxicity tests were conducted. The first extractions were with activated carbon to remove oil and grease. The water was then split by filtering through 0.45- and 0.7-µm filters and further treated to remove metals (by chelation extraction) and organics (by solid-phase extraction). This procedure enabled the pollutant phase causing the toxicity to be identified: particulate bound pollutants, filterable metals, or filterable organics.

The EPA TIE protocols consist of three levels of confirmation (EPA 1991a). These methods were designed for analyzing wastewater effluents; however, they have been used for stormwaters, sediment pore waters, and whole sediments (e.g., EPA 1991d; Bailey et al. 2000; Werner et al. 2000; Vlaming et al. 2000; USGS 1999; Burgess et al. 1997; Ho et al. 1999; Kosian et al. 1999; Boucher and Watzin 1999). Usually, only Phase I is conducted due to the time and expense required. The TIE Phase I is a physical and chemical fractionation process that separates chemical groups by their properties. The principal groups of contaminants include pH-sensitive and volatile compounds (such as ammonia), metals, and nonpolar organics. This consists of exposing Ceriodaphnia dubia neonates and/or P. promelas larvae to water fractions for 48-hour periods. If toxicity is removed in any fraction, subsequent chemical analyses can be used to confirm the removal of compounds which may be contributing to toxicity. These methods are relatively complex and should be conducted by an experienced laboratory.
Examples of Identifying Stressors

Diazinon was shown to be the primary toxicant in stormwater samples using C. dubia (Ohio EPA 1987). Anderson et al. (1991) compared numerous stormwater outfalls in the lower San Francisco Bay, CA. They found that nonpolar compounds in the most toxic stormwater (from a small, heavily industrialized drainage area) were the most important components of the toxicity, with lesser effects associated with suspended solids, metal chelates, and cationic metals. In another toxic stormwater study (from large parking areas surrounding an airport and industry), toxicity was most strongly influenced by cationic metals. Diazinon and chlorpyrifos in urban stormwater showed additive toxicity to C. dubia in a TIE (Bailey et al. 1997). TIE evaluations in the Sacramento–San Joaquin River basins confirmed that several organophosphate and carbamate pesticides were responsible for acute toxicity to C. dubia in water samples (Werner et al. 2000; Vlaming et al. 2000; Bailey et al. 2000). A TIE of pore water from a stormwater detention pond using C. dubia 48-hour exposures showed ammonia to be the primary toxicant, with some effects from metals (Zn, Fe, and Cu). The high level of ammonia may have obscured the metal toxicity (Wenholz and Crunkilton 1995).

Jirik et al. (1998) also used selected Phase 1 TIE studies to identify the toxicants most responsible for stormwater toxicity in the Santa Monica Bay area. Sea urchin fertilization tests
indicated EC50 values of stormwater of about 12 to 20%. Santa Monica Bay receiving waters were also found to be toxic, with the level of toxicity generally corresponding to the amount of stormwater in the receiving water. EDTA addition removed virtually all of the toxicity, implying that divalent metals were the likely toxicant component. Spiking studies showed that zinc, and sometimes copper, were the most likely metallic toxicants. Further studies, using EDTA vs. sodium thiosulfate for toxicity removal, also strongly implicated zinc as the likely cause of toxicity.

In situ tests also provide an excellent means for identifying the source and nature of the stressor by simply altering the exposure via chamber design and placement. It is essential to relate organism responses (e.g., mortality) with their correct, realistic exposure, such as overlying water, surficial sediment, or deeper sediments and pore waters (Figure 6.159). Useful in situ approaches to separating media effects and characterizing contaminant sources, pathways, and effects include characterization of benthic communities, in situ toxicity testing, and groundwater/surface water interactions (Greenberg et al. 2000; Figure 6.160). In a simplistic TIE approach, stressors can be partitioned out: overlying water, bulk sediment, interstitial water, light, suspended solids, flow velocity, and predator effects (Burton and Moore 1999) (see also Chapter 5). Strategic placement of chambers at reference and potentially impacted sites can identify both natural and anthropogenic stressors. Placement along known or suspected contamination gradients can provide an exposure–response relationship when combined with physicochemical measurements. For example, utilization of naturally occurring gradients (e.g., within and beyond a mixing zone) may facilitate an exposure–response characterization and regression analysis rather than a paired comparison (e.g., ANOVA) (Liber et al. 1992).

Useful in situ chambers for assessing stormwaters and surficial sediments are shown in Figures 6.161 through 6.164. Chambers are also buried in surficial sediments to assess sediment and groundwater associated contamination (Figures 6.165, 6.166, and 6.168) where chamber mesh...
windows contact surficial sediments (bottom tray) or overlying water (top tray). Test organisms are placed within the chambers during low flow (Figure 6.169). Following organism addition, high flow guards (aluminum sheet metal) are attached to stakes to protect the chambers (Figures 6.163 and 6.164).

Assessments of PAH-contaminated sediments have demonstrated why both laboratory and field toxicity exposures were essential to adequately identify key stressors and characterize exposure dynamics (Ireland et al. 1996; Sasson-Brickson and Burton 1991; Stemmer et al. 1990). Sediment-associated toxicity increased in the laboratory exposure of *P. promelas, C. dubia, D. magna,* and *H. azteca* as compared to *in situ* exposures, whereas toxicity decreased in overlying waters (Figure 6.156). Photoinduced toxicity from PAH and UV interactions and sampling-induced artifacts accounted for these laboratory-to-field differences. Toxicity was also reduced significantly in the presence of UV light when the organic fraction of the stormwater was removed. Photoinduced toxicity occurred frequently during low flow conditions, but was reduced during high turbidity associated with high flow conditions. Toxicity was also higher in overlying waters near the contaminated sediment surface as opposed to waters several centimeters above the sediment–water interface.

An elevation in temperature of Des Plaines River water accentuated the toxicity of the water and of sediments, using both water column and benthic species (Brooker and Burton 1998; Burton and Rowland 1999; Lavoie and Burton 1998). Responses were replicated in laboratory, *in situ,* and
Figure 6.165  *In situ* chamber used as a “peeper” (buried for pore water exposure) or sediment–water interface (half-buried) exposure.

Figure 6.166  *In situ* sediment–water interface chambers buried.

Figure 6.167  *In situ* chambers optimized for surface water and photoinduced toxicity effects from PAHs and UV light.

Figure 6.168  Chambers for conducting sediment bioaccumulation studies.

Figure 6.169  Loading *in situ* chambers that are peepers for sediment–water interface exposures.
artificial, side-stream exposures. The laboratory exposures helped define exact threshold temperatures, critical exposure times, and interactions with ammonia (Figure 6.170). Field exposures, on the other hand, better defined real-world exposures and interactions with other stressors, such as suspended solids and fluctuating temperatures. Conclusions based on laboratory exposures would have underestimated stream effects.

An urban site receiving large loadings of residential, commercial, and industrial stormwater runoff was assessed using an integrated low and high flow assessment (Moore and Burton 1999). The effects of turbidity and flow were shown by reducing the mesh size in the \textit{in situ} chambers (Figures 6.171 and 6.172). A survey of sediment quality during baseflow conditions found one depositional area where sediments were acutely toxic and contained elevated levels of contaminants. An \textit{in situ} toxicity assessment found that low flow water was not toxic, but high flows were toxic, and suspended solids and flow contributed significantly to overall stress. However, indigenous communities appeared to be affected more strongly by contaminated sediments than high flow conditions.

Newer TIE methods include whole-sediment manipulations, exposure to UV (Kosian et al. 1998), or \textit{in situ} exposures with various stressor partitioning methods and substrates (Burton et al. 1998; Greenberg et al. 1998; Moore and Burton 1999), and may reduce the likelihood of artifacts.

\textbf{Figure 6.170} Temperature threshold determination in the presence of contaminated site water and sediment vs. control waters and sediment. Survival (%) of \textit{Hyalella azteca}.

\textbf{Figure 6.171} Relationship between toxicity and suspended solids/flow. \textit{In situ} exposures in chambers with smaller mesh sizes decreased solids and flow and increased survival.
Toxicant Sampling and In-Stream Modeling Considerations

When sampling for, or predicting the fate of, toxicants, it is helpful to consider whether the likely contaminants tend to sorb to particulates, such as suspended solids or bedded sediments, or whether they tend to remain dissolved. Though metals will sorb to sediments in most waterways, if the water pH is acidic or if suspended colloids and solids concentrations are low, metals may remain in the water column. Dissolved metals do not necessarily equate with toxicity, as they may be complexed (e.g., carbonates, hydroxides) in less toxic forms. Many organics can be transported in dissolved forms at low suspended solid concentrations (EPA 1986). Adsorption can be predicted by knowing the octanol-water coefficient ($K_{ow}$), the organic carbon content of the suspended sediment, and then calculating the partition coefficient ($K_p$) (EPA 1986), as shown in Figure 6.173 (Novotny and Olem 1994). The $K_p$, however, is a site-specific value which varies at the site spatially and temporally during storm events and should thus be used with caution.

Sediment resuspension (scour) is an important mechanism affecting water column concentrations of many problematic constituents that tend to accumulate in stream sediments (especially pathogens, toxicants, and nutrients). Scouring of sediments can also be an important factor influencing water
turbidity in some cases. Methods for measuring sediment scour were discussed previously in this chapter in the general habitat discussion. In that case, the significant role that scour has on habitat was stressed. The measurement methods described there (used in conjunction with sediment quality information) can also be used to measure the resuspension of contaminated sediments. Modeling of sediment resuspension can only be crudely predicted because site-specific details are rarely available in sufficient detail and local scour “hot spots” (small areas where the flowing water has excessive shear stress) are extremely difficult to predict. However, scour around bridge piers has been investigated for several thousand years, and there are methods to reduce sediment losses in those situations. In most cases, it is only possible to grossly predict average sediment resuspension based on average stream bed conditions. Therefore, careful scour measurements should be conducted to indicate likely sediment resuspension rates for different flows for specific streams.

Many organic toxicants move in and through an ecosystem being controlled primarily by one fate process. Volatilization controls the fate of compounds such as trichloroethylene, toluene, xylene, acetone, and benzene. Adsorption dominates the fate of polychlorinated biphenyls, dioxins, and furans. For many common contaminants, such as the metals, metalloids, polycyclic aromatic hydrocarbons, and nutrients, multiple processes (e.g., biodegradation, methylation, photolysis, hydrolysis) dominate at different stages in different microenvironments.

A number of stream models exist for predicting pollutant fates, ranging from simple to complex, which may in limited cases be useful tools for stormwater effect studies. A summary of screening approach data requirements for metals and organics are listed in Tables 6.61 and 6.62, respectively.

Contaminants may move from their source through the receiving system, (e.g., stream, lake, wetland), in a conservative or nonconservative manner depending on the fate processes that dominate in that system and are characteristics of that particular toxicant. Generalized toxicant concentration profiles shown in Figure 6.174a reflect stream dilution and toxicant decay. This profile is not representative of reactive (nonconservative) constituents, such as highly volatile compounds, nutrients, species, or dissolved oxygen concentrations. Effects from these stressors must always be considered when toxicant fate and effects are being assessed. As shown in Figure 6.174b, during high flow conditions, contaminated sediment scour may increase concentrations in some stream segments before dilution and first-order decay profiles return. By constructing suspended solids profiles at low and high flow conditions, both sources and erosion- and scour-related stressors (e.g., sorbed toxicants and nutrients, oxygen demand, solids-related filter/gill clogging, or suffocation) can be better defined (see Figure 6.175).

Table 6.61  Summary of Data Requirements for Screening Approach for Metals in Rivers

<table>
<thead>
<tr>
<th>Calculation Methodology Where Data Are Used</th>
<th>Data</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hydraulic Data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Rivers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River flow rate, Q</td>
<td>D, R, S, L</td>
<td>An accurate estimation of flow rate is very important because of dilution considerations. Measure or obtain from USGS gauge.</td>
</tr>
<tr>
<td>Cross-sectional area, A</td>
<td>D, R, S</td>
<td></td>
</tr>
<tr>
<td>Water depth, h</td>
<td>D, R, S, L</td>
<td>The average water depth is cross-sectional area divided by surface width.</td>
</tr>
<tr>
<td>Reach lengths, x</td>
<td>R, S</td>
<td>The required velocity is distance divided by travel time. It can be approximated by Q/A only when A is representative of the reach being studied.</td>
</tr>
<tr>
<td>Stream velocity, U</td>
<td>R, S</td>
<td></td>
</tr>
<tr>
<td>2. Lakes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic residence time, T</td>
<td>L</td>
<td>Hydraulic residence times of lakes can vary seasonally as the flow rates through the lakes change.</td>
</tr>
<tr>
<td>Mean depth, H</td>
<td>L</td>
<td>Lake residence times and depths are used to predict settling of absorbed metals in lakes.</td>
</tr>
</tbody>
</table>
Table 6.61 Summary of Data Requirements for Screening Approach for Metals in Rivers (Continued)

<table>
<thead>
<tr>
<th>Source Data</th>
<th>Calculation Methodology Where Data Are Used*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Background</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal concentrations, $C_T$</td>
<td>D, R, S, L</td>
<td>Background concentrations should generally not be set to zero without justification.</td>
</tr>
<tr>
<td>Boundary flow rates, $Q_U$</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>Boundary suspended solids, $S_U$</td>
<td>D, R, S, L</td>
<td>One important reason for determining suspended solids concentrations is to determine the dissolved concentration, $C$, of metals, based on $C_T$, $S$, and $K_p$. However, if $C$ is known along with $C_T$ and $S$, this information can be used to find $K_p$.</td>
</tr>
<tr>
<td>Silt, clay fraction of suspended solids</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Locations</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>2. Point Sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locations</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>Flow rate, $Q_w$</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>Metal concentration, $C_{tw}$</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>Suspended solids, $S_w$</td>
<td>D, R, S, L</td>
<td></td>
</tr>
<tr>
<td>Depth of contamination</td>
<td></td>
<td>For the screening analysis, the depth of contamination is most useful during a period of prolonged scour when metal is being input into the water column from the bed.</td>
</tr>
<tr>
<td>Porosity of sediments, $n$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density of solids in sediments (e.g., 2.7 for sand), $\rho_s$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal concentration in bed during prolonged scour period, $C_{T2}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Derived Parameters**

<table>
<thead>
<tr>
<th>Derived Parameters</th>
<th>Calculation Methodology Where Data Are Used*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partition coefficient, $K_p$</td>
<td>All</td>
<td>The partition coefficient is a very important parameter. Site-specific determination is preferable.</td>
</tr>
<tr>
<td>Settling velocity, $w_s$</td>
<td>S, L</td>
<td>This parameter is derived based on suspended solids vs. distance profile.</td>
</tr>
<tr>
<td>Resuspension velocity, $W_{rs}$</td>
<td>R</td>
<td>This parameter is derived based on suspended solids vs. distance profile.</td>
</tr>
</tbody>
</table>

**Equilibrium Modeling**

<table>
<thead>
<tr>
<th>Equilibrium Modeling</th>
<th>Calculation Methodology Where Data Are Used*</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water quality characterization of river:</td>
<td>E</td>
<td>Equilibrium modeling is required only if predominant metal species and estimated solubility controls are needed.</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended solids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total organic carbon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other major cations and anions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* D = dilution (includes total dissolved and adsorbed phase concentration predictions); R = dilution and resuspension; S = dilution and settling; L = lake; E = equilibrium modeling. 

Table 6.62 Summary of Data Requirements for Screening Approach for Toxic Organics in Rivers

<table>
<thead>
<tr>
<th>Data</th>
<th>Methodology Where Data Are Used</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Hydraulic Data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate, Q</td>
<td>D, DA, DAK</td>
<td>An accurate estimate of flow rate is very important because of dilution, which for many organics is the most important process that influences their fate. Measure or obtain from USGS gauge.</td>
</tr>
<tr>
<td>Cross-sectional area, A</td>
<td>D, DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Water depth, h</td>
<td>DAK</td>
<td></td>
</tr>
<tr>
<td>Reach lengths, x</td>
<td>DAK</td>
<td></td>
</tr>
<tr>
<td>Stream velocity, U</td>
<td>DAK</td>
<td></td>
</tr>
<tr>
<td>Source Data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Background</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxicant concentrations</td>
<td>D, DA, DAK</td>
<td>Concentrations of organic toxicants may be negligible in areas not influenced by man.</td>
</tr>
<tr>
<td>Boundary flow rates</td>
<td>D, DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Boundary suspended solids</td>
<td>DA, DAK</td>
<td></td>
</tr>
<tr>
<td>2. Point Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locations</td>
<td>D, DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Flow rates, Q_w</td>
<td>D, DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Total toxicant concentration, C_T</td>
<td>D, DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Suspended solids, S_p</td>
<td>DA, DAK</td>
<td></td>
</tr>
<tr>
<td>Partition Coefficient and Rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant Data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficult to calculate accurately.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dye studies (as discussed earlier) are recommended in waste load allocation (WLA), or total maximum daily load (TMDL) studies to study point source mixing, movement of conservative pollutants, and to construct ambient toxicity profiles (EPA 1986; Figure 6.176). Multiple samples on a transect are necessary immediately downstream of sources or in wide streams (Figure 6.177). Samples of effluent from point sources (e.g., sewer overflow, culverts, tributaries [months], and stormwater) should be collected prior to dye studies, and both acute and chronic toxicity should be measured using EPA-recommended species (i.e., *Pimephales promelas*, *Ceriodaphnia dubia*), key surrogates (e.g., *Hyalella azteca*, *Selenastrum capricornutum*), and/or important resident species (e.g., trout). The dilution required to reach the no-observable-effects level (NOEL) in the toxicity tests should be the final sample points for constructing the dye isopleth (Figure 6.178; EPA 1986). These data may then be used to guide station location selection for ambient toxicity sample collection. In this manner, toxicity decay or persistence can be defined for various flow conditions.

SUMMARY

As indicated in many discussions in this book, multiple approaches are needed to effectively evaluate receiving water impacts in urban areas. This chapter presents details in collecting information pertaining to different ecosystem components and specific beneficial use impairments, including rainfall and flow monitoring; soil characteristics; aesthetics, litter, and safety; habitat
conditions; water and sediment chemical analyses; microorganism evaluations; benthos, zooplankton, and fish collecting; and tests for toxicity and bioaccumulation. This information supplements the information provided in Chapter 5 concerning collecting samples and selecting an experimental design. Chapter 7 briefly presents some statistical analyses tools, while Chapter 8 presents data interpretation for the complete study.
It is essential that there be an accurate description of the system’s hydrodynamics when assessing the effects of stormwater runoff on receiving waters. Flow represents the pollutant loading mechanism, and its power and frequency of occurrence can degrade the physical habitat. Instantaneous flow can be measured using traditional current meters, while long-term flow monitoring is usually conducted using stage recorders. Tracer methods are also useful, especially where the flows are quite shallow and the stream channel very rough. Tracers can also be used to effectively indicate diffusion and transport of pollutant discharges into small streams. Flow is also of primary consideration in supporting aquatic life, as minimum depths and velocities are needed for their survival. With urbanization, flow changes can be dramatic, with excessive flows occurring during wet periods and significantly reduced flows occurring during dry months.

The role that different rains have on wet weather-related receiving water effects is also important to understand through evaluation of local data. As an example, small rains (less than about 0.5 in in the upper Midwest) are important because they are associated with the majority of runoff events and they frequently exceed heavy metal and bacteria objectives, although they only account for a small fraction of the annual pollutant discharges. Intermediate-sized rains (from about 0.5 to 1.5 in in the upper Midwest) account for the majority of the pollutant discharges and subject the receiving waters to frequent high pollutant loads and moderate-to-high flow rates. Larger rains (from about 1.5 to 3 in in the upper Midwest) produce relatively small amounts of the annual pollutant discharges, but produce the most damaging flows from a habitat destruction standpoint. The largest rains are critical from a drainage aspect and must be controlled to provide safe conditions for inhabitants of the watershed. These rains must be controlled in the primary drainage systems, while excessive flows that exceed the capacities of these systems must be safely controlled in secondary systems.
drainages (such as temporary flooding of some roads, parking areas, vacant fields, etc.). Therefore, the type of receiving water problem being addressed is likely associated with a specific set of rain conditions, typically much smaller than the rains used in the design of storm drainage.

Soils can play a significant role in watershed and receiving water assessments. Most of the particulates being transported in stormwater originate as local soil, and their texture can have dramatic effects on stream turbidity levels and the amounts of erosion from nonpaved areas. In addition, soils in urban areas undergo significant modifications and are generally greatly compacted compared to natural soil profiles. The compacted soils provide much less infiltration for the rain water, increasing the runoff flow rates. Soil surveys can describe the soil types, textures, depths, chemical quality, and amounts of compaction, which are all useful measures. Soil modifications to enhance infiltration, to capture pollutants during percolation above the groundwater, and improve the fertility of the soil to enhance plant growth with minimal fertilization can therefore be important stormwater control practices.

Aesthetics, litter, and safety are all critical receiving water attributes that need to be quantified to indicate if basic beneficial uses (such as noncontact recreation) are being met. Many municipalities currently suffer large litter accumulations along public streams that significantly detract from their use and respect. Habitat problems are probably some of the most important impairments to aquatic life beneficial uses. Unfortunately, “standards” for habitat goals are not likely to become possible, requiring local investigations to compare receiving waters to local reference conditions. The role that highly fluctuating flows have on habitat is beginning to be understood. The amount of large woody debris, and other channel-forming materials, can be directly measured in streams, along with the rate of channel enlargement. Stormwater controls can possibly be designed to overcome habitat problems if the role of the causative impairment factors in local waters is better understood.

Water quality measurements also need to be made in a comprehensive receiving water assessment. Historically, most studies overly relied on expensive water quality measurements, with little supportive information. Currently, many areas are almost totally eliminating water quality analyses in stream assessments and only examining several basic stream biological conditions. As noted in this book, it is important that a balanced set of parameters be included in an effective program, requiring a basic set of traditional, plus specialized water quality measurements. The specific water quality parameters to be monitored should be selected based on the beneficial uses of the stream, along with additional indicator parameters that can identify the presence of inappropriate discharges and other unusual conditions. This chapter describes different field monitoring options, along with modifications that may be needed for conventional laboratory methods to be most effective for stormwater samples. Needed detection limits, along with safety and complexity, are presented as the most important factors that determine the most appropriate analytical methods that should be used for the selected parameters.

Microorganism measurements are needed in most receiving water assessments, especially in areas having water-contact recreation and consumption of aquatic life beneficial uses. Newly available microorganism measurement methods and changes in guidance on target organisms require a reexamination of traditional approaches in the assessment of these important parameters in receiving waters.

Benthos sampling is one of the most important measurements in receiving water assessments (along with habitat evaluations). Much guidance is now available on obtaining and evaluating appropriate samples. Fish sampling, although more complex to conduct and evaluate, is an important assessment tool, especially when relating to beneficial uses that are easier for the interested public to understand. Currently accepted methods for benthos and fish sampling are described in detail in this chapter and in related appendices.

Toxicity and bioaccumulation measurements can be important tools, especially when trying to identify cause-and-effect relationships between different stressors and receiving water impacts. Recently developed in situ toxicity test methods are especially useful tools because they subject the test organisms to natural conditions, such as fluctuations in receiving water conditions, and to...
the toxicity effects of in-place sediments. Traditional and newly developed methods for toxicity testing is presented in this chapter.

Chapter 6 presents a wide range of tools for characterizing many different components of ecosystems. Case studies also illustrate these procedures and show how they can be effectively utilized. Summaries of the advantages and disadvantages of the different methods are also frequently presented. Several appendices also present supportive information for the techniques given in this chapter.

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