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Assessing Biological Effects from Highway-Runoff Constituents

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A Contribution to the
National Highway Runoff Data and Methodology Synthesis

U.S. Department
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Assessing Biological Effects from Highway-Runoff Constituents

By DENNY R. BUCKLER and GREGORY E. GRANATO

A Contribution to the
National Highway Runoff Data and Methodology Synthesis

Northborough, Massachusetts
1999

U.S. DEPARTMENT OF THE INTERIOR
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PREFACE

Knowledge of the characteristics of highway runoff (concentrations and loads of constituents and the physical and chemical processes that produce this runoff) is important for decision makers, planners, and highway engineers to assess and mitigate possible adverse impacts of highway runoff on the Nation’s receiving waters. In October, 1996, the Federal Highway Administration and the U.S. Geological Survey began the National Highway Runoff Data and Methodology Synthesis to provide a catalog of the pertinent information available; to define the necessary documentation to determine if data are valid (useful for intended purposes), current, and technically supportable; and to evaluate available sources in terms of current and foreseeable information needs. This paper is one contribution to the National Highway Runoff Data and Methodology Synthesis and is being made available as a U.S. Geological Survey Open-File Report pending its inclusion in a volume or series to be published by the Federal Highway Administration. More information about this project is available on the World Wide Web at http://ma.water.usgs.gov/fhwa/runwater.htm

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SI* (MODERN METRIC) CONVERSION FACTORS

APPROXIMATE CONVERSIONS TO SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km
		AREA		
in^2	square inches	645.2	square millimeters	mm^2
ft^2	square feet	0.093	square meters	m^2
yd^2	square yards	0.836	square meters	m^2
ac	acres	0.405	hectares	ha
mi^2	square miles	2.59	square kilometers	km^2
		VOLUME		
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft^3	cubic feet	0.028	cubic meters	m^3
yd^3	cubic yards	0.765	cubic meters	m^3
NOTE: Volumes greater than 1000 l shall be shown in m^3.				
		MASS		
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams (or "metric ton")	Mg (or "t")
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5(F-32)/9 or (F-32)/1.8	Celcius temperature	°C
		ILLUMINATION		
fc	foot-candles	10.76	lux	lx
fl	foot-Lamberts	3.426	candela/m^2	cd/m^2
		FORCE and PRESSURE or STRESS		
lbf	poundforce	4.45	newtons	N
lbf/in^2	poundforce per square inch	6.89	kilopascals	kPa

APPROXIMATE CONVERSIONS FROM SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
mm	millimeters	0.039	inches	in
m	meters	3.28	feet	ft
m	meters	1.09	yards	yd
km	kilometers	0.621	miles	mi
		AREA		
mm^2	square millimeters	0.0016	square inches	in^2
m^2	square meters	10.764	square feet	ft^2
m^2	square meters	1.195	square yards	yd^2
ha	hectares	2.47	acres	ac
km^2	square kilometers	0.386	square miles	mi^2
		VOLUME		
mL	milliliters	0.034	fluid ounces	fl oz
L	liters	0.264	gallons	gal
m^3	cubic meters	35.71	cubic feet	ft^3
m^3	cubic meters	1.307	cubic yards	yd^3
		MASS		
g	grams	0.035	ounces	oz
kg	kilograms	2.202	pounds	lb
Mg (or "t")	megagrams (or "metric ton")	1.103	short tons (2000 lb)	T
		TEMPERATURE (exact)		
°C	Celcius temperature	1.8C + 32	Fahrenheit temperature	°F
		ILLUMINATION		
lx	lux	0.0929	foot-candles	fc
cd/m^2	candela/m^2	0.2919	foot-Lamberts	fl
		FORCE and PRESSURE or STRESS		
N	newtons	0.225	poundforce	lbf
kPa	kilopascals	0.145	poundforce per square inch	lbf/in

* SI is the symbol for the International System of Units. Appropriate rounding should be made to comply with Section 4 of ASTM E380.

Assessing Biological Effects from Highway-Runoff Constituents

By Denny R. Buckler *and* Gregory E. Granato

Abstract

Increased emphasis on evaluation of nonpoint-source pollution has intensified the need for techniques that can be used to discern the toxicological effects of complex chemical mixtures. In response, the use of biological assessment techniques is receiving increased regulatory emphasis. When applied with documented habitat assessment and chemical analysis, these techniques can increase our understanding of the influence of environmental contaminants on the biological integrity and ecological function of aquatic communities.

The contaminants of greatest potential concern in highway runoff are those that arise from highway construction, maintenance, and use. The major contaminants of interest are deicers; nutrients; metals; petroleum-related organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylene (BTEX), and methyl *tert*-butyl ether (MTBE); sediment washed off the road surface; and agricultural chemicals used in highway maintenance.

Hundreds, if not thousands, of biological endpoints (measurable responses of living organisms) may be either directly or associatively affected by contaminant exposure. Measurable effects can occur throughout ecosystem processes across the wide range of biological complexity, ranging from responses at the biochemical level to the community level.

The challenge to the environmental scientist is to develop an understanding of the relationship of effects at various levels of biological organization in order to determine whether a causal relationship exists between chemical exposure and substantial ecological impairment. This report provides a brief history of the evolution of biological assessment techniques, a description of the major classes of contaminants that are of particular interest in highway runoff, an overview of representative biological assessment techniques, and a discussion of data-quality considerations.

Published reports with a focus on the effects of highway runoff on the local ecosystem were reviewed to provide information on (1) the suitability of the existing data for a quantitative national synthesis, (2) the methods available to study the effects of highway runoff on local ecosystems, and (3) the potential for adverse effects on the roadside environment and receiving waters. Although many biological studies have been done, the use of different methods and a general lack of sufficient documentation precludes a quantitative national synthesis on the basis of the existing data. The Federal Highway Administration, the U.S. Environmental Protection Agency, the U.S. Geological Survey, the Intergovernmental Task Force on Monitoring Water Quality, and the

National Resources Conservation Service all have developed and documented methods for assessing the effects of contaminants on ecosystems in receiving waters. These published methods can be used to formulate a set of protocols to provide consistent information from highway-runoff studies.

Review of the literature indicates (qualitatively) that highway runoff (even from highways with high traffic volume) may not usually be acutely toxic. Tissue analysis and community assessments, however, indicate effects from highway-runoff sediments near discharge points (even from sites near highways with relatively low traffic volumes). At many sites, elevated concentrations of highway-runoff constituents were measured in tissues of species associated with aquatic sediments. Community assessments also indicate decreases in the diversity and productivity of aquatic ecosystems at some sites receiving highway runoff. These results are not definitive, however, and depend on many site-specific criteria that were not sufficiently documented in most of the studies reviewed.

INTRODUCTION

Biological effects are an important component of studies of the effects of highway runoff on water quality. The sensitivity of biota to changes in water quality, coupled with a high degree of public interest in their welfare, as well as legal requirements, make consideration of biological effects a high priority. The Clean Water Act (Federal Water Pollution Control Act of 1972, 33 USC §§ 1251 et seq.) and its amendments and revisions represent the primary Federal legislative actions directed toward the protection and restoration of the physical, chemical, and biological integrity of the Nation’s waters. More recently, the States have been integrating biological criteria into the development and enforcement of their water-quality standards (Davis and others, 1996). The intended use of biological criteria is to evaluate the effects of anthropogenic activities on the biological integrity and ecological function of aquatic communities. Because of these factors, biological assessment of the effects of nonpoint-source pollution has gained increased regulatory and scientific attention in recent years.

Background

The complexity of mixtures of chemical contaminants has, in part, lead to an increased regulatory emphasis on biological endpoints (using the responses of living organisms as indicators of contaminant presence and effects). Chemical measurements commonly do not allow prediction of the complex interactions that can occur in biological organisms. Scientific advances in the development and application of biological evaluation procedures also have lead to increased use of biological endpoints. These advances are a result of technological improvements in the area of environmental toxicology and chemistry. The early history of aquatic toxicology was a period in which the biological responses of organisms in the field were used to assess the extent of aquatic pollution. During this period, analytical chemistry methods for measuring environmentally relevant concentrations of contaminants were available for relatively few compounds. At this stage in the development of the science, the organism provided the most sensitive measure of environmental contaminants.

Over the past 15 to 20 years, major advances in analytical chemistry have made it possible to measure very low concentrations of many contaminants in environmental samples. These technological advances stimulated a period in which the regulation and monitoring of environmental contaminants were driven largely by the measurement of chemical concentrations in water, soil, or organisms. Water-quality criteria were developed for a wide range of contaminants by using information from laboratory toxicity studies to estimate acceptable environmental concentrations, but enforcement of water-pollution regulations was based largely upon the measurement of chemicals. During this period, efforts were made to develop models to predict biological effects from highway-runoff chemistry data and regulatory drinking water and aquatic life standards (Horner and Mar, 1985). It was recognized, however, that these planning-level estimates were specific to the area for which they were developed. Monitoring and assessment programs also were based on measuring chemical residues in water, sediments, and biota. Although these individual chemical-based approaches have served as the foundation for advancements in point-source pollution control, they also have drawn attention to the difficulties associated with collecting, analyzing, and interpreting the significance of complex mixtures of contaminants that can now be measured.

The most recent trend in environmental pollution assessment has been to return to the use of biological responses as measures of pollutant effects. However, current approaches usually are coupled tightly with analytical chemistry for measuring pollutant exposure. This integration of the biological and physical sciences is particularly important in the arena of nonpoint-source pollution, where the goal is to understand the environmental significance of complex mixtures of diverse arrays of contaminants. In this manner, the scientist or regulator can take advantage of current capabilities to measure low levels of myriad environmental contaminants, while relying upon data from the biota to integrate the cumulative biological effects of such exposure.

Purpose and Scope

The purpose of this report is to survey tools and techniques commonly used for the assessment of the biological effects from contaminants in stormwater runoff that are available for the study of highway runoff, and other sources of nonpoint-source pollution. This report describes the types of contaminants that are of particular interest to the study of highway runoff, discusses an array of biological assessment techniques that are appropriate for use in evaluating the effects of these contaminants, and evaluates data-quality issues for biological assessment techniques. A review of reports that focus on the effects of highway runoff on the local ecosystems provides information on (1) the suitability of the existing data for a quantitative national synthesis, (2) available methods to study the effects of highway runoff on local ecosystems, and (3) information about the potential for adverse effects on the roadside environment and receiving waters.

FACTORS FOR ASSESSING BIOLOGICAL EFFECTS

Factors of particular interest in the study of highway runoff are logically those that arise from construction, maintenance, and use of the highway system. Asphalt, concrete, rock, and steel are the primary highway-construction materials. Road oils, deicers, abrasives, solvents, paints, and vegetation-control agents are the primary highway-maintenance materials. Highway use results in the introduction of metals, fuels, combustion products, and toxic chemical spills as potential environmental contaminants. Finally, physical habitat disturbance resulting from construction, maintenance, and use of the highway system may also affect biota. General descriptions of the major classes of the factors associated with these activities are provided below.

Contaminants of Interest

Many of the contaminants normally associated with runoff from the Nation's highways have the potential for biological effects. Different contaminants will have varying biological effects depending on the physical and chemical properties of each constituent, the concentrations found in an environmental system, the sensitivities of organisms to adverse physical and chemical characteristics of the runoff, and the ability of the system and the individual organism to assimilate a given constituent or a given mixture of constituents. Highway-runoff contaminants of particular interest throughout the United States include deicers, nutrients, metals, industrial/urban-organic chemicals, sediment, and agricultural chemicals from industrial, commercial, residential, agricultural, and highway sources.

Deicers are chemicals used to remove ice and snow from roadways in the winter. Typically, salts such as sodium chloride and(or) calcium chloride are used. However, other compounds such as calcium magnesium acetate (CMA) are sometimes used in areas where the inorganic salts are a perceived problem. Loads of deicing chemicals per area of the road surface can be substantial on an annual basis, and they can change the geochemical conditions normally found in local receiving waters (Granato, 1996; Bricker, 1999).

Nutrients are constituents that are assimilated by organisms and that promote growth. In water-quality studies, the term generally applies to a number of measured constituents that contain nitrogen or phosphorus. Depending on the geochemistry of the runoff and the receiving water, nitrogen may occur as a nutrient in water as nitrite or nitrate anions, the ammonium cation, and(or) a number of natural organic compounds (Hem, 1992; Bricker, 1999). Phosphorus may occur as phosphoric acid, anionic forms including orthophosphate, or as a component of organic compounds. Phosphorus is generally believed to be the limiting nutrient in many aquatic systems because natural geochemical processes (Hem, 1992; Bricker, 1999) more readily control phosphorus compounds. Loads of these nutrients and resultant effects on aquatic biota from stormwater can be substantial (Grizzard and others, 1980).

Metals are considered ubiquitous contaminants today, but were much less abundant on the surface before man began to disturb the Earth's crust. There are a variety of biological mechanisms for sequestering and managing metals in the body because many metals are essential nutrients. The blood and tissues have a variety of carrier proteins, such as metallothionein (Suzuki, 1982), that regulate required inorganic nutrients, and metals generally show less tendency to accumulate in the body than many organic compounds. Metals, however, can pose important environmental concerns. One mechanism by which inorganic forms of metals tend to cause toxicological problems is known as isomorphic substitution—a process through which one element substitutes for another but may not be able to serve the same function. Organometallics, such as methylmercury, may interact with organisms in a manner that is similar to organic contaminants. Highway-related sources of metals include mobilization by excavation; vehicle wear; combustion of petroleum products; historic fuel additives, such as lead (Pb); maintenance materials, such as salts and deicers; abrasives; and catalytic-converter emissions.

Organic compounds in highway and road runoff that are derived from exhaust, fuel, lubricants, asphalt, and other synthetic chemicals are recognized as an environmental concern in many highway- and urban-runoff studies conducted over the last 20 years (Lopes and Dionne, 1998). These compounds are generally classified as semivolatile organic compounds (SVOCs) and volatile organic compounds (VOCs). Petroleum hydrocarbons, oil and grease, and polycyclic aromatic hydrocarbons (PAHs) are generally classified as SVOCs. Mono-aromatic petroleum compounds and the fuel additive Methyl *tert*-butyl ether are generally classified as VOCs.

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that can be formed during incomplete combustion of a variety of organic substances, including petroleum. Although some PAHs are manufactured for specific uses, such as in pharmaceuticals, the source of most environmental PAHs is from natural or anthropogenic combustion. This group of compounds includes more than 100 individual chemicals that are usually found as complex mixtures in the environment (Agency for Toxic Substances and Disease Registry, 1995). As their name implies, chemicals in this group are composed of more than one aromatic ring. PAHs are of relatively low molecular weight and generally are volatile and relatively hydrophobic, with a tendency to accumulate on airborne particulate matter or to associate with aquatic sediments. Common examples of individual compounds in this group include naphthalene, anthracene, phenanthrene, chrysene, and benzo(a)pyrene.

The mono-aromatic compounds benzene, toluene, ethylbenzene, and xylene (BTEX) are common constituents of crude oil and petroleum products. They also are produced as industrial solvents and as precursors for pesticides, plastics, and synthetic fibers (Harwood and Gibson, 1997). The primary sources of BTEX compounds relative to highway pollution are from spills and leakage of gasoline and other petroleum products. Similar to the PAHs, the BTEX compounds generally are volatile and relatively hydrophobic, but tend to be associated with storm-runoff sediments and ground-water contamination.

Methyl *tert*-butyl ether (MTBE) is an oxygenate compound that is added to gasoline to improve combustion and to reduce the levels of carbon monoxide emissions from automobiles. The total emission of MTBE from all sources into the environment in the U.S. in 1992 was estimated to be 3 million pounds (U.S. Environmental Protection Agency, 1994). Levels can be expected to increase as a result of the 1990 Amendments to the Clean Air Act, which require the addition of oxygenates to gasoline sold in urban areas that are not meeting target levels for carbon monoxide emissions. In a summary of urban stormwater in 16 cities and metropolitan areas, MTBE was the 7th most frequently detected VOC. MTBE was detected in about 7 percent of the 592 stormwater samples collected from the 16 cities and was detected at a rate of from 22 to 66 percent of samples taken in places where MTBE was in current use as a gasoline additive (Delzer and others, 1996). MTBE is quite volatile and would be expected to dissipate rapidly from soil or water surfaces (U.S. Environmental Protection Agency, 1993a). However, MTBE is about 40 times more soluble than the BTEX compounds and is less biodegradable than many common gasoline hydrocarbons. As a result, it is expected to be comparatively more persistent in ground water and in the shallow, fast-moving streams that are typical of urban and highway-runoff conveyances (Delzer and others, 1996). MTBE has been found in ground-water supplies at levels in excess of 200 milligrams per liter (mg/L) in some locations (U.S. Environmental Protection Agency, 1993b), posing a potential exposure risk to humans and aquatic life.

Sediment comprises inorganic and organic material and can be transported by, suspended in, or deposited by stormwater. Suspended sediment is generally considered to be one of the most substantial nonpoint-source contaminants (Waters, 1995; Crawford and Mansue, 1996). Studies across the Nation have documented that sediment can have large effects on the biology of receiving waters, ranging from the burial of fish eggs to the destruction of the entire aquatic food chain (Waters, 1995; Simmons, 1993). Many contaminants, including some metal ions, organic chemicals, and nutrients, are transported by sediment (Crawford and Mansue, 1996). Sediments have been associated with the destruction of aquatic habitat and a decrease in aquatic populations. Even relatively moderate sediment loading to an otherwise healthy stream can reduce the variety and abundance of aquatic life (Waters, 1995; Crawford and Mansue, 1996; Simmons, 1993). Sediment loads can also cause engineering problems by decreasing the capacity of channels and impoundments.

Although they receive only limited use in vegetation control for highway maintenance, herbicides and other agricultural chemicals are frequent components of nonpoint-source pollution. When a specific product is used intentionally for highway-maintenance activities, it is a relatively straightforward process to estimate the risk associated with the use of that product. Regardless of the actual sources, herbicides, pesticides, and other agricultural products frequently occur in surface-water runoff and should be given consideration in evaluations of nonpoint-source pollution.

Other Factors

Although outside the scope of this report, other factors may influence the health and abundance of individual organisms and biotic communities at a study site, such as spills of hazardous substances, physical habitat disturbance, and thermal pollution. Other factors of particular interest to the study and interpretation of apparent biological effects of highway-runoff quality are contamination and habitat disturbance caused by periodic highway construction and maintenance, hazardous substance spills, and other construction/development in the study area. Knowledge of the potential biological effects caused by these factors is important to assess results of a study to be included in a national or regional characterization of highway runoff. The effects caused by a spill or habitat disturbance from upstream development could potentially overshadow effects caused by highway-runoff discharges into receiving waters.

The high concentrations of chemicals caused by episodic spills of fuel, lubricants, coolant, and other chemicals are not normally considered to be characteristic of highway-runoff constituent concentrations. Spills, however, should be documented because a spill can affect measured water quality, and can affect biota in receiving waters. Unlike vehicle emissions or chemicals that are intentionally applied during highway construction and maintenance, the entry of contaminants into the environment from spills of hazardous substances is much less predictable. Large amounts of a wide array of hazardous materials are routinely transported on the Nation’s highways. Examples include fuels, agricultural chemicals, industrial compounds, and hazardous-waste products. About 2,400 chemical spills on the Nation's highways are reported to Federal authorities each year (National Response Center, 1999), and about 7,000,000 traffic accidents are reported by police to the National Highway Safety Administration each year (Cerrelli, 1998, 1997). McNeill and Olley (1998) noted the effects of small "routine spills"caused by traffic accidents on highway-runoff water quality, and upon stream biota at sites in their study area, and concluded that runoff best-management practice (BMP) structures should be designed to retain these small spills for cleanup. Because both minor and major spills can bias interpretations about the quality of highway runoff and the effects of highway runoff on biota, such events should be tracked and noted as explanatory variables for the data set collected at a given site. Once an accident occurs, the volume and types of contaminants that were released can usually be readily defined and appropriate measures for evaluating the nature and extent of the problem can be identified.

Although it is not specifically an environmental-contaminant concern, the potential for physical habitat disturbance in a highway-runoff study area is of great interest for site selection. Sedimentation and soil erosion, loss and changes in vegetation, physical habitat alteration, and disturbance of wildlife transport corridors are all potential concerns. Some of the most substantial biological changes caused by development are directly or indirectly related to altered hydrology. Despite efforts to use BMPs to attenuate the hydrologic effects of development, increased peak flows and more flashy runoff will cause physical modifications to the channel shape, bed substrate, and banks of receiving waters, with corresponding effects on aquatic habitat and biota. Loss of forest canopy, increases in paved area, and shallow and(or) muddy detention areas also may cause thermal pollution problems, which can exacerbate chemical stressors on aquatic organisms in receiving waters. All these factors will vary from site to site, and will affect interpretation of cause-and-effect relations between highway-runoff quality and the health and abundance of aquatic organisms in receiving waters.

BIOLOGICAL ASSESSMENT TECHNIQUES

The presence of measurable quantities of contaminants in the environment is an indicator of potential exposure. Contaminant presence alone, however, does not necessarily indicate the occurrence of deleterious biological effects. Measures of exposure must be linked to measures of effect in order to establish a causal relationship. Biological responses to contaminant exposure are the result of a progression of events that can be described as follows: The contaminant must first be released into the environment. This may be the result of intentional discharge, emission, or application. Contaminant input may also be due to unintentional releases such as spills, leakages, or other accidents. A variety of physical, chemical, and biological processes then come into play to determine the ultimate distribution, longevity, availability, and chemical form of the contaminant in the environment. The first interaction of the contaminant with an organism occurs at the biomolecular level. If the degree of exposure is sufficient to elicit a biochemical response, then there is the potential for effects at the tissue, organ, whole-organism, population, and community levels of biological organization.

Each level of biological organization has a resiliency, or assimilative capacity, that allows it to mitigate injury. In order for a contaminant to elicit an effect at the higher levels of organization (e.g., population or community levels), it must first exceed the ability of the lower levels to attenuate the response. For example, a 10 percent inhibition of an enzyme may or may not cause an observable response at the tissue, organ, or whole-organism level. The loss of a finite number of individuals may or may not be observable as an adverse effect on population status for some species. And finally, due to redundancy of function, the decline or loss of some populations may, or may not, observably affect the community if other species are able to fill those functional voids.

These relationships and interdependencies provide both promise and challenge to the use of biological responses as indicators of environmental health. Biochemical changes are the first biological responses that occur, therefore, they may be useful as early warning signals of contaminant problems. Effects at the biochemical level, however, may or may not result in measurable responses at the higher levels of biological organization. At the other end of the spectrum, many different physical, chemical, or ecological stressors can elicit apparently similar changes at the community level. As a result, it is often difficult to establish cause and effect at the higher levels of organization. The challenge facing the environmental scientist is to use the comparative specificity of responses at the lower levels of biological organization to help establish causal relationships with the more ecologically meaningful community levels. Measurement of effects at several levels of biological organization can provide a better understanding of the significance of a contaminant's presence, and can help determine whether a causal relationship exists.

In the remainder of this section, a variety of biological assessment techniques are discussed. No attempt has been made to provide an exhaustive review. Literally hundreds, if not thousands, of biological endpoints may be either directly, or associatively, affected by contaminant exposure. The techniques discussed below should be viewed simply as examples of biological tools that have been shown to be useful in evaluating environmental contaminants. Each technique has advantages and drawbacks that must be carefully considered in terms of its suitability to meet information needs for local, regional, and national studies. A number of the techniques are applicable to on-site use in receiving waters, whereas others are applied in the laboratory to samples collected in the field. In most situations, habitat assessments and analytical chemistry determinations should be made and documented in concert with biological measures to maximize opportunities for establishing cause and effect.

Biochemical, Physiological, and Histological Techniques

The principle underlying the use of subcellular biological indicators is that in order to elicit a toxic response, a chemical must first reach and interact with its biomolecular site of action. Whether that receptor site is a pore in a membrane, a transport protein on a fish gill, an enzyme in the liver of a bird, or any of a host of other possibilities, toxicity is always initiated on a molecular basis. Toxicants can interact with organisms on the molecular level in a wide variety of ways that challenge the maintenance of homeostasis (physiological stability). Metals can bind to various proteins disrupting membrane integrity, ion transport, and cellular metabolism. For example, mercury can bind to sulfhydryl groups on structural proteins (Donaldson, 1980), thereby disrupting membrane integrity. Copper (Cu) can interfere with ion regulation in fishes by affecting gill ATPase activity (Lorz and McPherson, 1976). Lead can deactivate δ-aminolevulinic acid dehydratase (ALAD), disrupting red blood cell metabolism (Goering, 1993). Organic contaminants also interact with organisms on the molecular level by disrupting membrane integrity and function, by displacing endogenous substrates of enzymes, or by diminishing energy reserves through increased demands on detoxification mechanisms. For example, pentachlorophenol can uncouple oxidative phosphorylation by interacting with the mitochondrial membrane (Moreland, 1980). Organophosphates and carbamates can displace acetylcholine and inhibit its hydrolytic cleavage by acetylcholinesterase (ACHe), thereby affecting nerve transmission (O'Brien, 1976). Widdows and Donkin (1991) report a variety of ways by which toxicants can diminish the energy reserves of an organism to the detriment of survival.

A wide range of biochemical and physiological parameters are known to be responsive to chemical exposure. However, relatively few of the measurable biochemical and physiological responses are specific to certain chemical classes (for example, ACHe inhibition by organophosphates and carbamates; ALAD inhibition by lead). The majority of responses are sensitive to a wide variety of chemical compounds and other environmental stressors. Thus, with a few exceptions, most biochemical and physiological measures are more useful as general indicators of organism health rather than specific indicators of individual chemical exposure. In order to establish cause and effect, responses that are general indicators of organism health must be used with other measures of chemical exposure, such as chemical residue analysis. The following descriptions address examples of biochemical, physiological, and histological techniques that have been shown to be useful in evaluating environmental contaminants.

Metal Sequestration and Regulation

Metallothioneins are a class of metal-binding proteins that are found in a wide variety of organisms (Roesijadi, 1992) that are inducible after exposure to Cadmium (Cd), Copper (Cu), Mercury (Hg), and Zinc (Zn) (Noel-Lambot and others, 1978). Metallothio–neins are thought to reduce the toxicity of heavy-metal exposure by sequestration (Hamilton and Mehrle, 1986), with toxicity occurring only after its binding capacity is exceeded (Brown and Parsons, 1978). Hepatic metallothionein levels in feral fishes from lakes contaminated with Cu, Zn, and Cd have been closely correlated to metals exposure (Roch and others, 1982). The inducible nature of metallothioneins suggests that they represent a regulatory mechanism and, as such, provide a useful indicator of exposure to certain metals. Other metal-binding proteins, such as ferritin (Fe) and copper-chelatin (Cu), have been less thoroughly studied in aquatic organisms.

Oxidative Metabolism

In organisms composed of more than one cell, oxidative metabolism is important for catabolic-energy production, reduction of oxygen radicals and maintenance of oxidative homeostasis within cells, and foreign compound (xenobiotic) metabolism. The cytochrome P450 monooxygenase (MO) system is one of the most extensively studied biochemical indicators of contaminant exposure and effects in fish and wildlife species (Stegeman and others, 1992). This inducible enzyme system has been shown to be responsive to a variety of environmental contaminants, including PAHs. The MO system plays a central role in detoxification of xenobiotic compounds by modifying their structure to a more readily eliminated form. The use of markers of oxidative metabolism in biological monitoring studies has been reviewed for aquatic organisms (Payne and others, 1987; Goksøyr and Förlin, 1992) and wildlife (Rattner and others, 1989). In feral organisms, a number of factors, such as age, sex, reproductive status, diet, disease, and general health conditions, can be confounding variables (Neal, 1980). However, successful quantitative use of MO activity in the field has been demonstrated by using caged organisms (Lindström-Seppä and Oikari, 1990) where factors affecting MO activity can be controlled or monitored during the period of exposure. An alternative means of controlling confounding factors is the use of in vitro systems. In these assays, extracts from environmental samples or SPMDs are tested in bioassays by using cell cultures. An example of such an in vitro model system for MO activity is the H4IIE hepatoma cell bioassay (Tillitt and others, 1991). In this bioassay, environmental contaminant extracts are used to dose the cells. After a period of incubation, ethoxyresorufin-o-deethylase (EROD) activity in the cells is measured. The MO response of the H4IIE cells is highly correlated to effects observed in the whole organism (Safe, 1987). Studies have demonstrated the ability of the H4IIE bioassay to be predictive of contaminant responses at higher levels of biological organization, such as egg mortality rates in PCB-exposed populations of fish-eating birds (Tillitt and others, 1992).

Reproductive Parameters

Reproduction is another physiological function that has been shown to be sensitive to contaminant exposure (Thomas, 1990). The complexity of the reproductive process and the confounding influence of behavior, nutritional status, seasonality, and other variables make it difficult to ascertain the effects of contaminants on the reproductive success in feral populations. However, a number of biochemical parameters associated with reproduction have been shown to be useful as indicators of contaminant exposure, including levels of vitellogenin and sex-steroid hormones (Thomas, 1990). Vitellogenin is the major yolk protein precursor in non-mammalian vertebrates. Under the influence of estrogen (van Bohemen and others, 1982), vitellogenin is synthesized in the liver and transported to the ovaries in the blood. Elevated levels of vitellogenin in male organisms have been linked to the presence of environmental contaminants (Folmar and others, 1996). Similarly, levels of sex-steroid hormones (for example, estrogen, testosterone, and precursors) have been shown to be related to reproductive status in fish (Mylonas and others, 1997) and have been shown to be responsive to a variety of environmental contaminants (Thomas, 1990; Folmar and others, 1996).

Histopathology

A wide variety of histopathologic changes in organisms can be used as indicators of contaminant exposure and effects. As with many other biological endpoints, confounding variables at the tissue and cellular levels include other environmental stressors, such as disease, parasitism, and normal seasonal variation (Hinton and others, 1992). However, certain tissues have received considerable attention as indicators of contaminant effects. Liver tissue is a primary target of interest as a result of its role in xenobiotic metabolism. Fatty infiltration, hepatocyte hypertrophy, vacuolation, and other hepatic effects have been related to contaminant exposure (Couch, 1975). Similarly, the kidney has received considerable attention (Meyers and Hendricks, 1985) because of its role as a site of elimination for many environmental contaminants. The gills of aquatic organisms are particularly sensitive to contaminant exposure because they serve as an initial and substantial site of uptake. Responses include epithelial hyperplasia, lamellar fusion, and general necrosis (Meyers and Hendricks, 1985; Jagoe and others, 1987). Finally, gonads (Hinton and others, 1992) and the immune system (Anderson and Zeeman, 1995) have been shown to be sensitive indicators of contaminant effects.

Tissue Analysis

Chemical analysis of contaminant concentrations in biological tissues is widely used as a measure of biological exposure to constituents of concern. Analysis of aquatic organisms' tissues, when used in concert with chemical analysis of the water column and bed sediments, provide a direct measure of the bioavailability of the constituents of concern. When these chemical data are used in conjunction with other biological indicators, effects on individuals and populations of aquatic biota can also be examined. Although measured constituent concentrations in runoff, sediment, and biota would appear to provide a quantitative measure of a cause-and-effect relation for biota, the geochemistry of the receiving waters will affect bioavailability from season to season and site to site (Bricker, 1999), and biological characteristics of the different taxa studied will determine accumulation rates (Crawford and Luoma, 1993). The complexity of the Nation's freshwater environments, as well as differences in surrounding land use, physical and hydrologic features, and variations in flow temperature and water quality from season to season, complicate the use of tissue analysis in a consistent manner on a national or regional scale (U.S. Environmental Protection Agency, 1986). Contaminant concentrations are comparable only within the same species, and for organisms at the same life stage, reproductive condition, size, weight, and sex (Crawford and Luoma, 1993). Also, different constituents are accumulated selectively by different tissues within each organism, so the type of tissue used for analysis is another factor for consideration. Therefore, the consistency of results depends upon a program design that accounts for these chemical, physical, and biological factors.

Many factors must be considered when evaluating use of tissue analysis in studies that are to produce data for potential application in local, regional, or national synthesis studies (Crawford and Luoma, 1993). These factors include: (1) measurable tissue concentrations that vary with environmental concentrations and exposure therein; (2) uptake of constituents that is relatively rapid in comparison to release; (3) tissue concentrations with low variability among individuals collected, to be representative of site conditions at a given time period; (4) availability of organisms that are hardy enough that can withstand contaminant concentrations of interest; (5) organisms that do not commonly travel beyond the immediate travel area; (6) organisms that are abundant and widespread in the study area to withstand repeatable harvest for analysis; (7) organisms that can be collected, and that are large enough to meet sample size requirements; (8) organisms that are easy to find and hardy enough to withstand captivity during controlled laboratory studies; and, (9) a species for which biological and toxicological information is available for comparison. Mollusks, fish, various aquatic invertebrates, and vascular plants have been successfully used in different applications for studies that include tissue analysis, and standard methods for the collection, processing, and analysis of organisms within these groups have been examined in terms of the suitability for a national synthesis of biological, sediment, and water-quality data studies (Crawford and Luoma, 1993).

Semipermeable Membrane Devices

The semipermeable membrane device (SPMD) is an in-situ water-sampling device that mimics the bioconcentration of organic contaminants in lipids of aquatic organisms (Huckins and others, 1993). Although not a biological assessment technique itself, it is a powerful sampling technique for sequestering environmental contaminants for biological assessments. The sampler operates passively and consists of a thin film of neutral lipid enclosed in a flat, semipermeable-membrane tube. Unlike living organisms that metabolize and excrete many toxic organic contaminants, often eliminating the causal link between residue concentration and adverse effects, these time-weighted sampling devices continue to concentrate organic contaminants from water to their maximum partitioning limits (exceeding 1 million fold for certain contaminants). Also, only biologically available contaminants concentrate in the sampler lipid because of the small size of the pores in the polymeric membrane. This selectivity contrasts with nearly all currently used water-monitoring techniques. The compounds taken up by the device can be analyzed by routine chemical analysis or be used as a source of exposure in biological assessment procedures. Data from SPMDs, however, must be calibrated in terms of the different equilibrium concentrations and the different rates of reaching equilibrium concentration for each constituent of interest.

Whole-Organism and Single-Species Techniques

Whole-organism, or single-species, toxicity tests have been widely used to evaluate the effects of environmental contaminants. Mortality, growth, and reproduction are the typical responses measured by toxicity tests. Additionally, whole-organism exposures are often used to obtain the biological samples required for the biochemical, physiological, and histological assessments described above. A summary of historical application of toxicity testing to highway runoff is provided by Smith and Lord (1990). Toxicity-testing procedures have been developed for a wide range of species, including microbes, algae, vascular plants, and aquatic and terrestrial invertebrates and vertebrates. Additionally, tests have been designed that can be conducted on-site or in the laboratory with field-collected samples. The choice of species and life stage to be tested and appropriate routes of exposure are important considerations in whole-organism testing. Species considerations include sensitivity to the contaminants being evaluated, availability of healthy specimens for testing, ease of culture and maintenance, and local importance and relevance to the geographic area being evaluated. Generally, early life stages of organisms are more sensitive to contaminant exposure. The routes of exposure evaluated in toxicity studies may include water, food, air, physical contact, and maternal transfer. The choice of exposure route depends upon the properties and environmental distribution profiles of the contaminants being evaluated. Appropriate environmental samples for assessment may include field-collected water and sediments or contaminant fractions sequestered through sampling procedures, such as the previously described SPMDs. Toxicity testing has many benefits, but it also has some limitations that should be considered as a component of a national or regional water-quality assessment (Elder, 1990). The following descriptions address examples of whole-organism toxicity-testing techniques that have been shown to be useful in evaluating environmental contaminants. Tests of toxicity that are associated with field-collected sediments are most effective when used in concert with other contaminant-evaluation procedures. The Sediment Quality Triad is one such method that combines the power of laboratory sediment-toxicity assessment, field measures of benthic invertebrate community structure, and analytical chemistry to evaluate potentially contaminated sites (Canfield, Kemble, and others, 1994).

Microbial Assays

Microtox® and Mutatox® are microbial assays that use bioluminescent bacteria (*Photobacterium phosphoreum*) to detect the presence of cytotoxic and genotoxic environmental contaminants. The assays can be used with unprocessed water samples or with extracts of various environmental media, including water, sediment, and tissue. They can also be used in conjunction with SPMD extracts. Microtox® is used to evaluate cytotoxicity by quantifying reduction in light output as a result of death of the bacteria. The assay has been shown to be sensitive to a wide range of toxicants (Kaiser and Palabrica, 1991; Jacobs and others, 1993). Mutatox® is similar in concept and procedure, but uses a dark mutant strain of *Photobacterium phosphoreum* to detect the presence of DNA-damaging chemicals. Genotoxicity of a sample is quantified by restoration of light production of bacterial cells upon reverting back to wild-type bacteria. It can be used with a similar suite of environmental media or SPMD extracts. The assay has been shown to detect genotoxicity with over 100 chemicals (Johnson, 1992a,b; Ho and Quinn, 1993). The relative ease and efficiency of the Microtox® and Mutatox® procedures make them ideally suited for screening large numbers of environmental samples.

Algal Assays

Algal growth studies have been used to evaluate the effects of environmental contaminants and nutrient enrichment on aquatic algal communities. The most commonly used species is the green alga *Selenastrum capricornutum*. A variety of responses can be measured, including optical density of exposed cultures, oxygen production and(or) carbon dioxide uptake, cell counts, gravimetric cell mass determinations, and measurement of chlorophyll (American Society for Testing and Materials, 1997e). Increases or decreases in these parameters, compared to control responses, are used to determine effects on algal growth.

Aquatic Invertebrate Assays

Aquatic invertebrates have been widely used to evaluate environmental contaminants that are in receiving waters and aquatic sediments. For evaluation of waterborne contaminants, tests with *Daphnia magna* and *Ceriodaphnia dubia* are most commonly used (American Society for Testing and Materials, 1997b,d). With both species of aquatic invertebrates, assessments of contaminant effects on survival, growth, and reproductive success can be made. Aquatic sediments are often chosen as a medium for toxicity testing because they are recognized as contaminant sinks. Methods for evaluating contaminants that are in sediments have been developed for use with several aquatic invertebrate species, including the amphipod *Hyalella azteca* and the midge *Chironomus tentans* (American Society for Testing and Materials, 1997f). As with the waterborne testing procedures, effects on survival, growth, and reproduction can be measured.

Early Life-Stage Toxicity Studies with Fish

Early developmental stages are often the most sensitive to chemical stressors (McKim, 1977). Toxicity studies can be conducted in which fish are exposed to environmental contaminants in water and(or) food, or by injection of environmental-contaminant extracts. Toxicity studies can be conducted with a wide range of fish species and can provide information on the short-term (acute) and long-term (chronic) toxicity of contaminants (American Society for Testing and Materials, 1997a,c). Egg injection studies are particularly useful for evaluating the effects of contaminants on early developmental stages. The procedure involves injection of contaminants into freshly fertilized eggs and subsequent evaluation of mortality, hatchability, and developmental effects (Walker and others, 1994).

In-Situ Toxicity Assessment

In-situ exposures provide a method for assessing the survival and health of organisms in waters from the geographic area under evaluation. Duration of these tests can extend from 96 hours to 30 days. Early life stages of fish and(or) other organisms are exposed directly to water from the site of interest, using environmental chambers at the site (Finger and Bulak, 1988; Hall and others, 1993). Measurements are made to evaluate effects on survival, growth, and behavior. This type of exposure study is particularly useful for evaluating pollution effects on species that are indigenous to the site of interest. Results from in-situ exposures provide information on the actual response of organisms to mixtures of contaminants as they occur in natural systems. When compared with laboratory toxicity studies with single chemicals, they can provide insight into the additive or synergistic properties of contaminant mixtures. Documentation of effects on survival and development of early life stages of fishes also allows extrapolation to population-level effects, providing valuable information for assessing the role of contaminants on declining populations.

Population and Community Techniques

Measures of contaminant effects at the population and community levels focus on the structural and functional properties of the biotic components of ecosystems. Population and community surveys provide direct measures of aquatic community structure and function. Biological surveys can range from evaluations of single indicator species to evaluations of large proportions of organisms found in an aquatic receiving system. Obviously, both the level of resolution and the associated cost of the evaluation increase with the level of complexity of the survey. Application of biodiversity assessment techniques to transportation projects is not fully developed but may yield valuable information to highway planners, scientists, and engineers involved in addressing environmental concerns (Bardman, 1997). Regional differences in species composition, habitat characteristics, thermal and hydrological regimes, and water-quality problems must be taken into consideration in the design of a nationally consistent ecological assessment program. Fortunately, however, a wide variety of sources of biological population and community information for many areas of the country is readily available from the USGS, the USEPA, the U.S. Fish and Wildlife Service, and many State wildlife and environmental protection agencies (Gurtz, 1994). Structural analysis techniques seek to determine the abundance and distribution of various taxa as indicators of population and community stress. Presence or absence, numerical abundance or biomass, reproductive and recruitment success, and spatial distribution of indicator species are used to determine biotic status (Petersen, 1986; Sheehan and others, 1986).

Functional analysis techniques focus more on the ecological processes of ecosystems, such as community respiration, nutrient cycling, decomposition rates, colonization rates, and trophic guild analysis (Cairns and others, 1972; Rapport and others, 1985; Crossey and LaPoint, 1988).

Well-designed studies, using population and community techniques, are a valuable component of water-quality studies (Cuffney and others, 1993a,b). Population and community techniques relate directly to ambient conditions in receiving waters, incorporate a large variety of exposure pathways, eliminate the need to culture and maintain laboratory test organisms, and incorporate secondary effects that arise from unnatural changes in predator/prey relations. These techniques, however, present a challenge in interpretation of the magnitude of effects caused by changes in water quality with respect to effects caused by different natural and anthropogenic factors that will also change the structure and abundance of different biota in an aquatic system.

Measurements of effects at the higher levels of biological organization are important in terms of clearly demonstrating the ecological relevance of environmental impairment. However, current techniques are not specific to contaminant stress. Other forms of habitat degradation, interspecific competition, and nutrient alteration can cause similar effects at the population and community levels. Therefore, detailed documentation of the characteristics of the local stream habitat is necessary (Meador, Hupp, Cuffney, and Gurtz, 1993). Physical characteristics of the receiving waters, such as the geomorphic channel unit (pool, riffle, or run), riparian vegetation, bank stability, water temperature, stream depth, current velocity, and bed-sediment particle-size distribution, will determine the natural ability of a stream reach to support a given population. Therefore, these factors should be assessed and documented upon site selection, should be measured concurrently with each assessment, and should be similar for control and study sites (Gurtz, 1994). Other natural factors such as extremes of flow, life cycles of aquatic taxa, and the accessibility of in-stream habitats, are important. The USGS National Water-Quality Assessment (NAWQA) Program does annual community assessment work in the summer/fall low-flow period to avoid sampling soon after flood events, to obtain samples of algae, aquatic invertebrates, and fish in a mature development stage, and to access stream sites when smaller streams and rivers can be waded safely (Cuffney and others, 1993a,b; Meador, Cuffney, and Gurtz, 1993; Porter and others, 1993; Gurtz, 1994).

In order to establish cause and effect, it is particularly important to apply population and community techniques in concert with analytical chemistry determinations and other biological measures of contaminant stress that may be more contaminant specific. Water temperature influences the metabolic and reproductive rates of algae, benthic invertebrates, and fish. Aquatic organisms are also sensitive to changes in dissolved oxygen, pH and alkalinity, and other water-quality properties and constituents. Therefore, an understanding of watershed geochemistry is important to interpretation of population and community data.

Different elements of biomonitoring serve separate but complimentary goals when population and community techniques are applied to different key assemblages, including algae, benthic invertebrates, and fish. These different taxonomic groups respond differently to natural or anthropogenic disturbances because of differences in habitat, food, mobility, physiology, and life history; thus, an approach that utilizes more than one assemblage adds information that can be used to develop cause-and-effect relations (Gurtz, 1994). Examples of population and community assessment techniques are described below.

Algal Population and Community Assessments

Algae are an important component of population and community assessments because they have a short life span (days to weeks), they readily exchange constituents with the water column and sediments, and reflect local water quality because they are sedentary (Porter and others, 1993; Gilliom and others, 1995). Algae are operationally defined by physical characteristics of appearance and size, and by the substrate upon which they thrive. Algal populations are typically characterized by the list of taxa present, by interpretation of the community structure, and by the algal biomass per unit area. The taxon-specific physiological requirements or tolerance for defined ranges of water-quality conditions are known for over 3,000 algal species; therefore, population and community data can be a good measure of water quality (Porter and others, 1993). Algal populations can be sampled from natural surfaces, or—for more consistency between sites—from artificial substrates emplaced in a receiving water. Even when the substrates are designed to closely mimic natural surfaces, the population structure may not represent the natural population in the surrounding habitat. This bias, however, may not be a liability when the study objective is to measure the effect of highway-runoff quality on algal populations at a number of sites. An example of protocols for design, sample collection, documentation, and QA/QC for population and community assessments of algae on a regional and national scale is provided by Porter and others (1993).

Aquatic Invertebrate Population and Community Assessments

Aquatic invertebrates are an important component of population and community assessments because these organisms have longer life spans (months to years) than algae, live in close association with streambed sediments, and are relatively sedentary and good indicators of local water quality (Cuffney and others, 1993a,b; Gilliom and others, 1995). Aquatic invertebrates are operationally defined as a diverse group of taxa that live in, on, or near streambed sediments, including aquatic insects, mollusks, crustaceans, and worms. Aquatic invertebrate populations are typically characterized by the list of taxa present, by interpretation of the community structure, by the biomass collected by standard methods within a predefined study area, and by visible deformities in the specimens collected. Aquatic invertebrates exhibit different tolerances to contaminants that are found in sediments and the water column (Hamilton and Saether, 1971; Hare and Carter, 1976; Wiederholm, 1984; Warwick, 1985). Some genera are relatively intolerant, and low-contaminant levels eliminate them from the benthic community, while other genera are more tolerant, and would only be affected or completely disappear at higher contaminant levels. Measures of the presence, absence, and abundance of various taxa can be used as an indicator of aquatic community status (Plafkin and others, 1989). As with fish population assessment, indices have been developed for assessing aquatic habitat quality by the use of aquatic invertebrate community metrics (Hilsenhoff, 1987). Additionally, deformities in chironomid larvae have been shown to be related to the presence of contaminants in aquatic sediments (Hamilton and Saether, 1971; Cushman, 1984; Wiederholm, 1984; Warwick, 1985, 1989). Reported deformities include thickening of the exoskeleton, enlargement and darkening of the head capsule, asymmetric mouth-parts, missing or fused lateral teeth, and deformed antennae. Canfield, Swift, and LaPoint (1994) provide a description of considerations regarding the use of benthic invertebrate assessments for evaluation of contaminated sediments. An example of protocols for design, sample collection, documentation, and QA/QC for population and community assessments of aquatic invertebrates on a regional and national scale is provided by Cuffney and others (1993a,b).

Fish Population and Community Assessments

Fish are an important component of population and community assessments because these organisms have long life spans (years to decades), are of interest to the public, are potentially economically valuable, are highly mobile, and so are indicative of long-term watershed health (Meador, Cuffney, and Gurtz, 1993; Gilliom and others, 1995). Fish exist as a diverse group of species with different preferences for instream habitat. Fish populations are typically characterized by the list of species present, by physical measurements of, and by visible anomalies on, the specimens collected. One of the more widely used methods for evaluating fish community assemblages is the Index of

Biotic Integrity (Karr, 1981; Karr and others, 1986). The scientist using the procedure utilizes a number of metrics, including such factors as species composition, trophic composition, and fish abundance and condition, to determine the structural and functional status of the fish community. Although the original index was developed for use in midwestern streams, alternative metrics have been developed for other regions of the country (Miller and others, 1988). An example of protocols for design, sample collection, documentation, and QA/QC for population and community assessments of fish on a regional and national scale is provided by Meador, Cuffney, and Gurtz (1993).

DATA-QUALITY CONSIDERATIONS

Data quality, compatibility, and utility are important considerations in biological assessments of environmental contaminants. Findings of an environmental assessment must also be readily transferable and useful to resource managers and regulators. To meet these objectives, supporting ancillary information must be available that (1) documents the methods and procedures that are used, (2) describes quality-assurance and quality-control procedures that are employed, and (3) adequately describes the environmental setting that is being evaluated in terms of geographic location, condition, and other confounding variables. Brief discussions of these topics are provided below; more thorough discussions are provided by Meador, Hupp, Cuffney, and Gurtz (1993); Meador, Cuffney, and Gurtz (1993); Gurtz and Muir (1994); and the Intergovernmental Task Force on Monitoring Water Quality (1995).

Documentation of Methods

Biological assessment techniques are continually being developed and refined. New techniques and improvements of existing techniques serve to enhance our understanding of the implications of environmental contaminants. However, this evolution of the science makes it increasingly difficult to compare data over time. This is of particular concern to long-term and broad-scale monitoring and assessment programs that draw upon the expertise of a wide range of scientists. The exclusive use of published and proven procedures would help alleviate this concern, but would impede scientific advancement. Environmental scientists can help resolve this dilemma by thoroughly documenting and describing any new techniques employed and, when practical, by conducting studies designed to compare the results of new and existing methods (ITFM, 1995). This will help to ensure temporal- and spatial-data compatibility and comparability while continuing to allow advancement of the science.

Quality Assurance and Quality Control

In terms of quality assurance and quality control, biological assessments provide a few unique challenges compared to chemical measurement of contaminant residues (USEPA, 1995). The goals are the same: to provide sensitive, reproducible, and transferable data. There are, however, differences in the ways in which certain performance criteria are addressed. The parameters that are typically evaluated as indicators of quality include accuracy, precision, bias, detection limits or performance range, interference, and matrix applicability. In analytical chemistry, these performance parameters can be addressed by the use of internal standards and spiked, blind, and blank samples (Jones, 1999). Directly comparable procedures do not exist, however, for many biological endpoints. Precision and bias can be addressed in the usual manner by subsampling, by repeated measures of an endpoint, and comparison of the results of different techniques. Accuracy, however, is more difficult to determine given the frequent lack of "true" values or applicable standards for biological endpoints. To indirectly address accuracy, environmental scientists draw upon comparisons of results obtained at reference sites, the use of reference toxicants, and an understanding of "normal" ranges of values for biological endpoints. Detection limits and performance ranges are commonly a function of the range of possible values for a biological parameter, rather than a measure of the ability of an instrument or procedure to detect a response. Finally, interference and matrix applicability considerations include variables, such as disease or other factors that can elicit similar biological responses, factors affecting contaminant availability, or other confounding environmental variables.

Description of Environmental Setting

A final consideration for assuring comparability and utility of the results of a biological assessment is an adequate description of the environmental setting for the site that is being evaluated. It is important to provide descriptive (or explanatory) information that precisely defines the geographic location of the sampling effort and that describes pertinent environmental variables that may affect the outcome of the assessment. An important factor to be considered in the design of biological assessments is the size of receiving waters. Important physical and biological characteristics change as small streams develop into large rivers. Therefore, it is important to compare biological characteristics among streams of similar size. Also, such factors as land-use practices in the watershed, the presence or absence of riparian vegetation, streambed substrate, geomorphological features, nutrient levels, and the natural geochemistry of the receiving water may influence the behavior of environmental contaminants and their availability to biota. Additionally, physical and hydrologic habitat characteristics can have a profound influence on the types and quantities of biota that are present. This is particularly important because an acceptable biological assessment technique must be used to discern between natural variability and the effects of anthropogenic influences in order to establish a cause-and-effect relationship. While these variables may seem obvious to the scientist performing the assessment, their documentation is essential to the ultimate users of the information (for example, resource managers, regulators, and the public).

Comparability Issues for a National Synthesis

Contaminants are only one of many possible environmental stressors that can affect the status of biota. The confounding variables identified above are important considerations in any attempt to perform a national synthesis or to build a national data base of contaminant effects. To be useful, the results obtained from a biological assessment must be scientifically comparable (among investigators and laboratories) and geographically transportable (across States or regions). As such, the availability of supporting information, as discussed above, is essential to the process. The isolated results of a toxicity test or a population assessment are of little value without this supporting information.

It would be difficult to rank each of the example techniques discussed in this report in terms of its value to a national assessment. In fact, each has its inherent strengths and weaknesses, and it is through the use of combinations of these techniques to provide multiple lines of evidence that they have their greatest utility. However, some general observations on the inherent strengths of the major categories can be made (table 1). Generally, as one moves from techniques applied at the lower levels of biological organization (biochemical level), to techniques applied at the higher levels (single species and community levels), such factors as chemical specificity and sensitivity tend to decrease. In contrast, responses observed at the higher levels of organization tend to have greater ecological relevance and regulatory utility. This relationship, once again, indicates the importance of using a suite of techniques applied at a variety of levels of biological complexity.

Table 1. Comparative strengths of categories of biological assessment techniques

Category	Relative cost	Relative sensitivity	Chemical sensitivity	Ecological relevance	Regulatory utility
Biochemical, physiological, and histological techniques	Low	High	High	Low	Low
Whole-organism and single-species assay techniques	Medium	Medium	Medium	Medium	High
Population and community techniques	High	Low	Low	High	Medium

BIOLOGICAL EFFECTS OF HIGHWAY-RUNOFF QUALITY: A LITERATURE REVIEW

Relevant journal articles and reports were reviewed as part of this study on potential biological effects from highway runoff. These publications were identified in the National Highway-Runoff Water-Quality Data and Methodology Synthesis (NDAMS) data base as having an emphasis on the biological effects of highways and highway runoff on local ecosystems (Granato, 1997). This review is intended to examine existing information in terms of the factors necessary for assessing biological effects, available biological assessment techniques, and data-quality considerations that may be useful to highway practitioners. This is not an exhaustive review of the available highway-runoff literature, nor is it a survey of the larger body of potentially relevant literature on the biological effects of urban runoff. This review, however, does represent a summary of reports that have a primary emphasis on biological effects of highway pollution that were readily obtainable in a 2-year literature search of much broader scope. Hopefully, the review will also provide information to assess current and future needs of highway practitioners in terms of the effects of highway runoff on local ecosystems.

Of the 44 articles and reports reviewed, 32 were data/interpretive reports, which documented original research, 8 were summary or literature review reports, 2 were abstracts from conference proceeding poster sessions, and 2 were general policy papers (table 2, at back of report). Of the 32 data/interpretive reports, 18 were from studies done within the United States, and the remaining 14 were from Canada and Europe. Within the United States, the potential effects of highway-runoff quality were examined on different types of ecosystems in California, Colorado, Florida, Louisiana, Maryland, North Carolina, Ohio, Vermont, Virginia, Washington State, and Wisconsin. Fieldwork, reported by all of these interpretive studies, spanned a 26-year period from 1970 through 1996. About 80 percent of the fieldwork was done before the end of 1985.

Biological studies commonly emphasize different species or taxonomic groups because of the different ways they respond to various environmental stresses. For example, differences in life span, mobility, and ecological niche among algae, aquatic invertebrates, and fish are useful for examining effects of contaminants at different temporal and spatial scales (Gilliom and others, 1995). Among the 32 data/interpretive research reports reviewed, algae were examined in 11; plants, 6; aquatic invertebrates, 16; fish, 9; amphibians, 1; earthworms, 1; and other organisms, 7. Within each group, different species were examined or used as test specimens in the various interpretive reports.

Typically, ecological effects of highway-runoff quality on receiving waters have been predicted using statistical models of runoff-contaminant concentrations and loadings based upon the event mean concentrations of monitored storms (Horner and Mar, 1985; Smith and Lord, 1990; Driscoll and others, 1990; Young and others, 1996). These predictive approaches, which compare modelled loads and concentrations to published regulatory limits, indicate that there should be no measurable effects at sites with annual average daily traffic (ADT) volumes below 30,000 vehicles per day (VPD) (Smith and Lord, 1990; Driscoll and others, 1990; Young and others, 1996). Biological assessments, however, have shown changes in individual organisms and community structures, even at sites with relatively low traffic volumes (table 2).

Although many studies have been done, the full significance of intermittent discharge of highway runoff on the ecology and quality of receiving waters is not well documented (Dupuis, Kaster, and others, 1985; Maltby, Forrow, and others, 1995; Dupuis and others, 1999). It is difficult to assess the effects of highway runoff in a natural setting because increases in ADT are associated with higher levels of background contaminant sources from surrounding land uses (Dupuis, Kobriger, and others, 1985).

Factors for Assessing Biological Effects

Biological effects associated with highway-runoff quality are dependent on the concentrations and availability of the constituents of interest in highway runoff, on the concentrations and availability in receiving waters, and on the long-term storage and availability in soils and sediments in the vicinity of the highway and in receiving waters. Concentrations and availability of constituents in highway runoff depend on the physical and chemical characteristics of the roadway, vehicular sources, precipitation, and deposition from background sources in the study area (Young and others, 1996; Irish and others, 1996). Concentrations and availability of constituents in receiving waters depend on dilution by receiving waters (Dupuis, Kaster, and others, 1985; Horner and Mar, 1985; Driscoll and others, 1990; Cooper and others, 1996), the physical and chemical characteristics of local receiving waters (Driscoll and others, 1990; Bricker, 1999), the magnitude of background sources (Dupuis, Kaster, and others, 1985; Shively, and others 1986; Davis and George, 1987), and biological uptake and processing in the local ecosystem (Birdsall and others, 1986; Baekken, 1994; Maltby, Boxall, and others, 1995; Cooper and others, 1996; Schafer and others, 1998). Field research and resultant highway-runoff quality models indicate that, in general, event mean concentrations of pollutants in runoff and receiving waters are not acutely toxic (Dupuis, Kaster, and others, 1985; Driscoll and others, 1990; Maltby, Forrow, and others, 1995; Dutka and others, 1998). However, studies also indicate that soils and sediments from highway runoff are a reservoir of contaminants that can affect the ecosystems near runoff discharge points (Portele and others, 1982; Gjessing and others, 1984; Mudre, 1985; Maltby, Forrow, and others, 1995; Boxall and Maltby, 1997).

Highway runoff contains a complex mixture of potentially adverse constituents. These constituents include deicers, nutrients, metals, organic chemicals, sediment, and potentially, herbicides and pesticides. Effects of these different contaminants depend upon study location, environmental setting, and the characteristics of the receiving waters (table 2). Cumulative effects on biological systems from highway-runoff quality include effects of all bioavailable contaminants and any interactions among them.

Deicers were noted as a contaminant of concern in 15 of the studies reviewed (table 2). Several studies specifically mentioned deicers as a potential problem for aquatic ecosystems (Corbett and Manner, 1975; Crowther and Hynes, 1977; Dickman and Gochnauer, 1978; Dupuis, Kaster, and others, 1985). Crowther and Hynes (1977), in a controlled experiment, noted a chronic effect defined as increased drift in streambed organisms in a freshwater trout stream when chloride concentrations exceeded 1,000 milligrams per liter (mg/L). Dickman and Gochnauer (1978) also did a controlled experiment by introducing 1,000 mg/L pure sodium chloride into an unpolluted stream during a 4-week experiment to detect changes in stream microbiota. This study indicated that, when compared to an upstream control station, the abundance and diversity of algae was suppressed by the salt, but bacterial density increased because of suppression of predator organisms. Adams-Kszos and others (1990) noted toxic effects in toxicity tests using bridge runoff when salt concentrations exceeded about 11,000 mg/L. They also noted possible synergistic effects between deicers and metals that caused toxicity in bridge-runoff dilutions. Deicers contain many major and trace elements, and can contribute a large percentage of total solute loads in highway runoff (Harned, 1988; Granato, 1996).

Metals, as environmental contaminants, were mentioned in 32 of the studies reviewed (table 2). In many studies, increased metal concentrations were detected in the tissues of animals and plants exposed to soils and sediments in highway environments (Gish and Christensen, 1973; Corbett and Manner, 1975; Dupuis, Kaster, and others, 1985; Birdsall and others, 1986; Davis and George, 1987; Baekken, 1994; Cooper and others, 1996; Dupuis and others, 1999). Although these studies did not define the toxicity of the metals to these organisms, several studies noted the potential for bioaccumulation of metals higher in the food chain (Gish and Christensen, 1973; Birdsall and others, 1986; Cooper and others, 1996).

The studies reviewed indicated biological accumulation of highway-related metals not typically studied in the United States, including antimony from automobile brakes (Reifenhauser and others, 1995) and the platinum group elements (PGEs) platinum, palladium, and rhodium that are associated with catalytic converters in exhaust systems (Helmers, 1996; Schafer and others, 1998). In addition to these German studies, PGEs have been measured in elevated concentrations in England (Pearce and others, 1997) and in San Diego, California (Hodge and Stallard, 1986). PGEs may be a concern for future highway research because of the widespread use of catalytic converters in automobiles, because PGEs have similar toxicities as copper, chromium, cadmium, nickel, and zinc (Kaiser, 1980), and because PGEs may be available as hydroxide or chloride complexes (Wood, 1991). PGEs, however, are not normally prevalent in aquatic ecosystems, and information about the chemistry and toxicity of these metals is relatively sparse.

Volatile and semivolatile organic compounds (VOCs and SVOCs, respectively) in urban and highway runoff are a major concern because measured concentrations can exceed national drinking water and aquatic life standards and guidelines (Lopes and Dionne, 1998). One or more of these organic compounds normally associated with vehicle exhaust, fuel, lubricants, and(or) asphalt were studied in 19 of the reports that were reviewed (table 2). The majority of highway-runoff investigations, done by the FHWA and the State-highway agencies, analyzed samples for volatile suspended solids (VSS), total organic carbon (TOC), and oil and grease. Studies that were conducted in the 1970’s and early 1980’s, however, did not define individual organic chemicals or the major classes of organics—VOCs, and SVOCs (including polycyclic aromatic hydrocarbons, PAHs)—in highway runoff, receiving waters, or biota (Dupuis, Kaster, and others, 1985; Smith and Lord, 1990; Driscoll and others, 1990). Many later studies of urban and highway runoff from the United States did detect PAHs at concentrations that exceed aquatic health standards (Lopes and Dionne, 1998). Several studies by a British research group found that PAHs were the most toxic of all the highway runoff constituents measured in highway-runoff sediments collected from a British stream (Boxall and others, 1993; Maltby, Boxall, and others, 1995; Maltby, Forrow, and others, 1995, Boxall and Maltby, 1997). A different European study found tissue enrichment of organic compounds in benthic invertebrates and fish that was up to five times higher than tissue concentrations at background sites (Baekken, 1994). A recent study in California (Cooper and others, 1996), however, did not detect PAHs in road runoff, receiving waters, and the tissue of aquatic biota at the detection limits of 5 parts per billion for the water samples and 2 parts per million for the tissue samples.

Sediment (solid material that has been transported from its place of origin by erosion) is viewed as a major nonpoint-source contaminant throughout the United States (Davenport and others, 1991). Sediment is considered a problematic contaminant because it physically disrupts aquatic habitats and acts as a transport mechanism for other pollutants in aquatic ecosystems (Waters, 1995). Suspended solids and sediment were examined as a potential contaminant in 5 of the studies reviewed, and sediment was examined as a matrix for transport, storage, and release of other potential contaminants in 17 of the studies reviewed (table 2). In one study, effects of sediment from highway construction along a high mountain stream reduced the abundance and diversity of aquatic biota at four of seven sites (Cline and others, 1982). The effects varied from site to site depending on the local depositional environment and hydrologic effects, such as the periodic scouring action of high stream flows at some sites in this high-gradient stream. In many studies, sediments were mentioned as a potential reservoir for metals (van Hassel and others, 1980; Wanielista and others, 1982; Shively and others, 1986) and for organic contaminants (Gjessing and others, 1984; Boxall and others, 1993; Lopes and Dionne, 1998). Ney and van Hassel (1983), concluded that highway-runoff sediments were a source of pollutants to biota in Virginia streams because fish species associated with sediments had higher metal body burdens than species associated with the water column.

Other constituents in highway runoff (table 2) that may adversely affect aquatic life include major ions (in six of the studies reviewed), oxygen demand and oxygen deficit (five studies), nutrients (seven studies), and herbicides/pesticides (four studies). Although major ions (and many trace elements and metals) are natural components of natural waters and necessary nutrients for aquatic organisms (Hem, 1992), a change in the chemical signature of the receiving waters can affect the local aquatic community (Bricker, 1999). For example, Corbett and Manner (1975) noted a change from calcium-carbonate type to sodium chloride-type waters in some receiving streams in Ohio. In another study, the release of sulphate in highway runoff changed nutrient dynamics and caused eutrophication in a small Vermont lake by scavenging the iron that had removed phosphate from the aquatic system (Morgan and others, 1984). Oxygen demand from point sources and urban areas dominated the biological changes in a study that included highway runoff as one source (Davis and George, 1987). The inhibitory effects of highway-runoff constituents seemed to attenuate biological oxygen demand in some laboratory experiments (Portele and others, 1982; Dupuis, Kaster, and others, 1985). However, both stimulatory and inhibitory effects of highway runoff have been recorded in the literature (Winters and Gidley, 1980; Dupuis, Kaster, and others, 1985; Dutka and others, 1998). Highway runoff supplies nutrients to receiving waters, but the effect of these nutrients may be attenuated by metals and other contaminants in stormwater (Grizzard and others, 1980). Potential problems with herbicides and pesticides were discussed in several studies, but these studies concluded that proper management and timing in the application of chemicals to the right-of-way should reduce or eliminate adverse effects (Kobriger and others, 1984; Kramme and Brosnan, 1985; Shively and others, 1986).

Biological Assessment Techniques

The biological assessment techniques recorded in the highway-runoff research included histopathological techniques in the form of tissue analysis of biological samples collected, bioassays in the form of field and laboratory toxicity tests, population and community assessments, and behavioral studies. Of the 44 reports reviewed, 19 reported results of tissue analysis (table 2). The toxicity of soil sediment or runoff was assessed in 25 different reports. Plants, algae, aquatic invertebrates, and fish of different species were used as test subjects in the different toxicity tests. Community assessments were an integral part of about 16 of the studies reviewed. The drift of benthic species in the stream environment was assessed in two separate studies. Biological studies were supported by chemical analysis of soil water or sediment in about 35 of the studies reviewed.

Use of tissue analysis is prevalent in the literature relating to highway-runoff quality. In many studies, tissue analysis indicates that contaminants in sediments and soils near the highway environment can be mobilized within the food chain (table 2). Several studies have detected uptake of contaminants into plant tissues, and in some cases, changes in the physical form and apparent health of plants at sites where sediment and soil had elevated levels of contaminants in the highway environment (Wanielista and others, 1982; Dupuis, Kaster, and others, 1985; McFarland and O'Reilly, 1992; Reifenhauser and others, 1995; Schafer and others, 1998). Gish and Christensen (1973) demonstrated potentially toxic concentrations of metals in earthworms collected from soils near highways. This may indicate the availability of these metals in soils or sediments that could be eroded by runoff during large storms. Ney and van Hassel (1983) noted that the amount of metal uptake depended upon fish species, the specific affinities of the different tissues within a fish, and site characteristics controlling the availability of metals in the local environment. Birdsall and others (1986) found that tissue concentrations of lead in tadpoles varied with differences in ADT among 20 sites in Maryland and Virginia. Generally, concentrations of different highway-runoff constituents in tissue samples collected from aquatic communities were elevated near highway outfalls when compared to background concentrations in the same species (van Hassel and others, 1980; Dupuis, Kaster, and others, 1985; Baekken, 1994; Maltby, Forrow, and others, 1995; Cooper and others, 1996).

Generally, bioassays of highway runoff do not indicate acute toxicity for aquatic life. In a number of algal assays, Winters and Gidley (1980) found both stimulatory and inhibitory effects. They attributed the inhibition to higher metal concentrations in those samples. Wanielista and others (1982), however, detected only stimulatory effects in algal assays. When Portele and others (1982) conducted assays on algae, zooplankton, and rainbow-trout-fish fry, they found that soluble contaminants affected the algae and zooplankton and that suspended solids affected the trout fry. In assays with heterotrophic microorganisms, algae, and salmon eggs, Gjessing and others (1984) did not detect acute toxicity in runoff from a two-lane Norwegian highway. Dupuis, Kaster, and others (1985) did field and(or) laboratory toxicity tests on a number of species, including a number of micro- and macroinvertebrates, algae, and fathead minnow. The field bioassays were done at three sites—two in rural Wisconsin and one in a rural watershed in North Carolina—with ADT counts below the predicted 30,000 VPD biological effect threshold. These sites were chosen to examine the effects of highway runoff without significant background contaminant loads (Dupuis, Kaster, and others, 1985). Dupuis, Kaster, and others (1985) tested undiluted highway runoff from melting snow near a rural highway with low traffic volume (7,400 VPD) and highway runoff from a busy urban freeway (120,000 VPD). Many of these laboratory bioassays, however, were done with filtered runoff, or with runoff that was slowly circulated from reservoir tanks in which sediments had settled. These tests did produce some sluggish behavior in the fish, but no measurable acute toxicity. High mortality in the invertebrates was accompanied by high mortality in control groups, so there was no clear indication of highway-runoff toxicity (Dupuis, Kaster, and others, 1985). The algal bioassays done by Dupuis, Kaster, and others (1985) included a number of treatments with different nutrients and the metal chelating agent EDTA. These tests did not indicate the toxicity of runoff but did indicate an inhibitory effect caused by the metals in solution. A series of reports from a study in England did not demonstrate acute toxicity of runoff or receiving waters, but did determine that PAHs in highway sediments could be toxic to benthic organisms exposed to these contaminants (Boxall and others, 1993; Maltby, Boxall, and others, 1995; Boxall and Maltby, 1997). In a review of different studies on the toxicities of different deicers, including sodium chloride and calcium magnesium acetate (CMA), McFarland and O'Reilly (1992) noted some toxic effects on plants, invertebrates, and freshwater fish at very high deicer concentrations but lesser effects at concentrations expected in receiving waters. Adams-Kszos and others (1990) indicated that bridge runoff was toxic to sunfish when deicing chemical concentrations were high. Dutka and others (1998) used several different standardized bioassay methods on bridge runoff from several storms in Ontario, Canada. None of the tests produced acute toxicity, but different tests indicated genotoxicity for different storms. Because of the differing results from storm to storm and from test to test, Dutka and others (1998) concluded that a battery of tests was necessary to characterize the toxicity of runoff at highway sites.

Population and community techniques generally indicated local differences between control sites and study sites near highway-runoff discharge points. Corbett and Manner (1975) found that when compared to control sites, areas affected by highway runoff had fewer sensitive species of aquatic plants and animals. Dickman and Gochnauer (1978) documented changes in stream microbiota that were caused by sodium chloride in a controlled experiment in an otherwise unpolluted stream. Cline and others (1982) documented reductions in density, abundance, diversity, and taxonomic structure at four of seven sites that were caused by sediment from highway-construction activities. Wanielista and others (1982) demonstrated that areas affected by bridge drains had fewer species than control areas. Dussart (1984), however, found that areas affected by road runoff had higher productivity of algal and filamentous organisms. Dupuis, Kaster, and others (1985) reported ambiguous results at low traffic sites. In this study, some sites receiving highway runoff showed no changes in abundance or diversity, but other sites, sometimes along the same highway, did show effects from the highway runoff. Mudre (1985) also reported some effects, but had ambiguous results on a new highway with low traffic volumes (ADTs of about 6,000 to 15,000 VPD). It was also demonstrated that the availability of pollutants at these sites was controlled by streamflow, organic carbon content of sediments, and the overland flow distance between the road and the stream (Mudre and Ney, 1986). Davis and George (1987) found that effects from urban runoff overshadowed the effects of highway runoff at several stations along a river with a rural-to-urban gradient in land use. Baekken (1994) discovered that the diversity and abundance of species were lower near a highway outfall than at background sites, but these effects were localized around the individual discharge points. Maltby, Forrow, and others (1995) documented loss of sensitive species and changes in the relative abundance of other species at four of seven sites downstream of a British highway. Cooper and others (1996) did not detect changes in fish and most invertebrates, but did note reductions in stonefly populations in some sites downstream of the highway. In highway-runoff studies, some of the more pronounced effects were apparent among benthic-invertebrate populations. This may be because benthic invertebrates have close associations with streambed sediments (Cuffney and others, 1993a,b), and different species have different and characteristic sensitivities to minor changes in water quality (Kennen, 1998; Newton and others, 1998). In the highway studies, however, differences in population and community structure may also have been partially attributable to other explanatory variables that caused changes between control and study sites (Smith and Kaster, 1983; Cooper and others, 1996).

Data-Quality Considerations

Careful study design and thorough data-quality documentation are considered necessary to overcome the large variabilities in time and space, as well as the large uncertainties that are inherent in studies of aquatic biology. For individual studies to be considered valid, current, and technically defensible (to meet the information needs of the highway community), it is necessary to document (1) methods, (2) quality assurance and quality control (QA/QC) information, (3) a thorough description of the environmental setting, and (4) effects of seasonality (Granato and others, 1998). It is also necessary to standardize protocols for implementing and documenting these four factors to combine existing data, or to design and implement a new data-collection program for regional and(or) national synthesis that will meet information needs and data-quality objectives (DQOs). For example, in a literature review designed to assess potential effects of contaminants from bridges, Dupuis and others (1999) noted that several studies did not provide sufficient documentation to support definitive conclusions for their National Cooperative Research Program study. To implement a regional and(or) national synthesis, it is also important to study enough sites to characterize the various cause-and-effect relationships that may be prevalent in different environmental settings.

Detailed documentation of study methods is necessary to assess existing data sets to ensure that the data reported in different studies are comparable and meet DQOs for a regional or national synthesis (Granato and others, 1998). Methods of sample and data collection, analysis, and interpretation were at least partially documented in 34 of the 44 reports reviewed (table 2). It is difficult, however, to sufficiently document methods with the detail necessary for complete repeatability of experimental results; detailed documentation can be voluminous. For example, Dupuis, Kaster, and others (1985) used 406 pages of text to describe sites, methods, and results for studies at two streams and one lake. This report also was supplemented with a 238-page report that described the methods used and the comparability among accepted methods (Dupuis, Kreutzberger, and others, 1985). Use of national protocols, therefore, may lead to consistent local, regional, and national data sets and will reduce the amount of documentation required in each report by establishing accepted methods at the national level.

Published protocols are useful in that they establish standard methods, document these methods, and provide economy in the presentation of study results. For example, USGS's National Water-Quality Assessment (NAWQA) Program has established standard methods for virtually every aspect of its biological assessment components, including site characterization (Meador, Hupp, Cuffney, and Gurtz 1993; Fitzpatrick and others, 1998), tissue analysis (Crawford and Luoma, 1993; Nowell and Resek, 1994; Hoffman, 1996), and community assessment techniques (Cuffney and others, 1993a,b; Meador, Cuffney, and Gurtz, 1993; Porter and others, 1993). Required methods and resultant protocols should be driven by program objectives; however, method documentation, adequate methods comparison, systematic data-base structures, and collaborative efforts among agencies collecting environmental data will open up many opportunities for use of existing and intensive biological assessment data bases (Gurtz and Muir, 1994; ITFM, 1995). For example, the State of New Jersey has a network of more than 700 stream sites used for macroinvertebrate community assessment that are distributed among different land-use categories in the six physiographic regions of the State (Kennen, 1998). This data set includes a number of minimally disturbed areas in each physiographic region that can be used as regional reference/control sites.

Documented methods are an important part of necessary quality-assurance programs, and quality-control documentation ensures that methods meet data-quality objectives. Therefore, both quality assurance and quality control (QA/QC) are important components of study design and documentation (Jones, 1999). Compared to performance standards for chemical laboratories, objective statements of method accuracies for biological field methods are not quantitative (ITFM, 1995). QA/QC factors typically reported for chemical studies such as precision, bias, performance ranges, interferences, and method detection limits, however, can be defined in the context of biological assessment methods if methods and protocols are well established and method comparability studies are properly documented. For example, Lenz and Miller (1996) compared five different aquatic-invertebrate sample-collection methods at six sites in Wisconsin and found that the equipment and methods used affect the number of and the relative abundance of different species collected.

Most of the highway studies with a biological component incorporated some form of QA/QC documentation because of the variability of biological systems; thus, the need for documented experimental controls is recognized. There was some mention of quality-assurance and quality-control measures in all but 12 of the 44 reports reviewed. Documentation of replicate samples was included in 21 of the reports reviewed (table 2). Use of control sites, or experimental control samples was mentioned in 16 of the studies reviewed. Use of experimental standards was documented in six studies. Other forms of QA/QC were documented in 11 studies. For biological studies, a critical component of the QA/QC and documentation process is proper selection of and complete characterization of the study sites.

In studies of the biological effects of highway-runoff quality, detailed descriptions of the environmental setting are necessary to interpret and standardize results. In most studies, the site characteristics were described as confounding factors that influenced study results. Cline and others (1982) identified site-hydraulic factors as the controlling variables that determined the effects of sediment on biota in a high-gradient mountain stream. In a study of benthic invertebrates, Smith and Kaster (1983) found that differences in streamflow and the composition of stream substrate could overshadow effects of runoff on population characteristics at sites receiving runoff from highways with low traffic volume (ADTs less than 10,000 VPD). In another study, tissue lead content in tadpoles increased with increasing ADT on different highways among 20 sites in Maryland and Virginia (Birdsall and others, 1986). In this study, however, analysis of the differences between sites indicated that seasonality, microtopography (erosive or depositional environments), the residence time and episodic flushing of water and sediments, and differences in site sediment characteristics introduced variability in the relation between ADT and lead concentrations in tadpoles. The site characteristics of topography (stream gradient), flow velocity, sediment organic matter, and the overland flow distance from the pavement to the stream controlled the availability of highway-runoff related metals in sediment at six stream sites near a new highway in Virginia (Mudre and Ney, 1986).

Descriptions of the study-site characteristics were inadequate for use in a quantitative national synthesis for most of the studies reviewed because this information must be assessed using constant methods and documented in consistent formats from site to site for regional or national interpretation. Dupuis, Kaster, and others (1985) fully documented the highway characteristics that they thought would be explanatory variables controlling the quality of storm-water runoff. These characteristics include traffic volume, highway type, number of lanes, existence of curbs, pavement material (concrete or asphalt), and the percent of paved and nonpaved area contributing to the highway-runoff drainage system. Dupuis, Kaster, and others (1985) also characterized many of the physical hydrologic features of the study sites, including historical average annual precipitation, precipitation and stream flow during the study period, watershed area, stream gradient, watershed land use, and other potential sources of point and nonpoint pollution that are commonly recognized as explanatory variables for biological studies. Many of the site characteristics necessary to compare results among sites, however, were not sufficiently documented in this or the other 44 highway-runoff reports reviewed.

To examine the effects of highway-runoff water-quality runoff on biological systems at different sites, it is necessary to document many site characteristics that can be used to relate habitat to physical, chemical, and biological factors. Factors not characterized by most highway studies include elevation, sinuosity (topographic channel pattern), active channel width (the stream width at bank-full discharge), channel conditions and features (such as runs, riffles, and pools), bank stability, water-management features, instream fish cover, canopy cover, and detailed descriptions of the dominant substrate (boulders, cobbles, gravel, sand, silt, and(or) mud). Detailed diagrammatic mapping of stream features is also considered necessary for interpreting results of bioassessment studies (Meador, Hupp, Cuffney, and Gurtz, 1993; ITFM, 1995; Fitzpatrick and others, 1998; Newton and others, 1998). To facilitate a national synthesis, other factors are also considered important, such as site ecoregion, location in latitude and longitude, classification in a nationally recognized system of stream-order number, watershed identification (such as the USGS hydrologic unit code), watershed soils, surficial and bedrock geology, physiography, potential vegetation, and upstream wetlands (Fitzpatrick and others, 1998). Fortunately, methods for site assessment and characterization are well documented and much of the large-scale information is available in existing geographic information systems if the latitude and longitude of a site is known (Meador, Hupp, Cuffney, and Gurtz, 1993; ITFM, 1995; Fitzpatrick and others, 1998; Newton and others, 1998). The USGS national bridge scour data base, with information about 27,000 bridge sites in 12 states, is also a good source of stream-site data, including local land use, stream width, surface profile, vegetation, bed and bank material, channel profile, depth, erosional environment, and location information (G.W. Parker, USGS, oral comun., 1999).

It is necessary to have sufficient and consistent data from enough different sites to characterize explanatory variables to interpret biological and water-quality data on a regional or national scale. Integration of biological and water-quality data is often hindered by lack of consistent data and the small number of sites for which both types of data are available (Kennen, 1998). Driscoll and others (1990) developed a highway-runoff quality model using precipitation, average annual streamflow, and total hardness of surface waters from predefined zones within the conterminous United States to predict pollutant loadings and ecological effects from highway-runoff quality at any given site. To determine the number of sites needed to establish a sufficient national data set, it is necessary to examine the problem in terms of the number of regions needed to quantify each of these explanatory variables. To define pollutant loadings, Driscoll and others (1990) used estimates of average concentrations from highway-runoff studies and estimated runoff volumes from 9 rainfall zones defined by average annual storm characteristics, including duration, intensity, volume, and antecedent dry period (which have been revised to 15 rainfall zones by the USEPA, 1992). Driscoll and others (1990) used 18 divisions of average annual streamflow to estimate dilution in receiving waters. These divisions were defined in a geometric progression from 0.05 to 5.00 cubic feet per second per square mile of drainage area, and when applied nationally, these divisions are used to define 30 zones in the conterminous United States. Driscoll and others (1990) estimate the geochemical availability and effect on aquatic organisms by pollutants in receiving waters using 6 divisions of hardness defined in 5 even increments from less than 60 to greater than 240 parts per million as calcium carbonate. This method of geochemical classification yields 24 different hardness zones within the conterminous United States. When these three variables are combined, they produce about 140 distinct zones of precipitation, dilution, and geochemistry within the conterminous United States. To characterize effects on biota, it is also necessary to characterize habitat features. The ecoregion approach is often used for classification of similar habitat features on a national scale, and about 76 different ecoregions are defined as individual zones within the conterminous United States (Omernik, 1995; ITFM, 1995). Superposition of these zones upon the zones of precipitation, dilution, and geochemistry defined, by Driscoll and others (1990), would greatly increase the number of distinct zones to be studied, and would still define site characteristics in a rather coarse manner.

Ecological systems are subject to variability in time, as well as to variability in space. Effects of seasonality were discussed in 16 of the 44 studies reviewed (table 2). There are two distinct approaches to the problem of seasonality. One approach is to control for seasonality by collecting all samples within prescribed seasonal conditions. This approach assumes that the biological community, if studied under similar conditions from year to year, will integrate adverse effects throughout the year. Investigations using this approach often involve sampling during summer low-flow conditions because aquatic organisms are at a good point in their life cycle for sampling and analysis, and the low flows facilitate sample collection in the streambed (M.R. Meador, USGS oral comun., 1999). Another approach is to attempt a detailed characterization of the effects of runoff during different seasons. For example, examination of the effects of deicing chemicals on aquatic ecosystems would be the driving factor for sampling during the winter in highway-runoff studies; in fact, 12 of the 16 studies that discussed seasonality also addressed deicers as a potential contaminant.

There are also long-term temporal considerations when evaluating effects of highway runoff on biota. Mudre and Ney (1986) demonstrated that the availability of metals (in highway-runoff sediment deposits) was a function of total annual precipitation. During one study in Massachusetts, differences in temperature and precipitation caused loads of deicing chemicals to vary from 50 percent to 200 percent of the median load over a 5-year period (Granato, 1996). Studies should be long enough to document potential effects and(or) interpret data within the context of long-term weather records in the study area (Averett and Schroder, 1994; Granato and others, 1998). Among the 32 data/interpretive reports reviewed, the duration of fieldwork at each site was greater than 1 year in 14 reports, was 1 year or less in 15 reports, and was not documented in 3 reports (table 2).

SUMMARY

Advancements in our understanding of the mechanisms through which contaminants exert their effects have stimulated interest in using biological techniques for environmental pollution assessment and monitoring. Although many biological endpoints have been shown to be responsive to contaminant exposure, relatively few are specific to individual chemicals. No single biological endpoint can fully define the significance of environmental pollution at a given site; each has its particular strength and weakness. Biochemical assays may be most useful for demonstrating direct effects from contaminants; studies with single species may help minimize confounding environmental variables; and population and community assessments may be the most appropriate techniques for estimating the ecological significance of contaminant perturbation. However, when used in conjunction with analytical chemistry and habitat assessment as multiple lines of evidence, biological assessments can help provide the cause-and-effect linkages between environmental pollution and ecological impairment.

A review of 44 reports on the biological effects of highway runoff on local ecosystems reveals several information gaps. The use of different methods from study to study and a general lack of sufficient documentation preclude making quantitative comparisons among different studies using the existing data. Qualitatively, the literature indicates that constituents from highway runoff and from highway-runoff sediments deposited in receiving waters near the highway are found in the tissues of aquatic biota, and that these sources may affect the diversity and productivity of biological communities, even though bioassays would suggest that highway runoff is not often toxic to aquatic biota. To provide the quantitative information needed, it is necessary to obtain information using standard methods, and to document study results in a manner that will be useful for a national or regional synthesis.

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TABLE

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water

[ADT, average daily traffic; BMP, Best-management practice; D/I, data/interpretive; NA, not applicable; ND, not discussed; P/A, poster/abstract;

Reference	Report type	Dates of data collection	Location/ environmental setting
Adams-Kszos and others, 1990	D/I	09/1983–05/1985	New York/Lake Chautauqua, Bridge scupper sampling
Baekken,1994	D/I	1991	Oslo, Norway/2-lane highway, high hardness Norwegian lake
Bardman, 1997	Policy	NA	Continental United States
Birdsall and others, 1986	D/I	07/1982–12/1982	Maryland and Virginia/highway, receiving waters, and control sites
Boxall and others, 1993	P/A	ND	ND/streams affected by highways
Boxall and Maltby, 1997	D/I	10/1993, 01/1994–04/1994	England/streams crossed by M1 motorway
Cline and others, 1982	D/I	1975–77	Rt. 14 Colorado/high mountain stream
Cooper and others, 1996	D/I	03/1995–05/1996	California/streams adjacent to roadways
Corbett and Manner, 1975	R/S	1975 (approx.)	Ohio/roadsides
Crowther and Hynes, 1977	D/I	12/1973–02/1975	Canada, Great Lakes region/rural “trout” stream, urban stream
Davis and George, 1987	D/I	1978–80	England/river on rural-to-urban gradient with sewage, urban, and highway inputs
Dickman and Gochnauer, 1978	D/I	07/1973–10/1973	Quebec, Canada/pristine stream draining humic marsh in a wilderness park
Dupuis, Kaster, and others, 1985	D/I	07/1981–10/1983 (each study done in sequence about 1 year in duration)	Wisconsin, North Carolina/highways in rural stream, lake and piedmont regions
Dupuis and others, 1999	R/S	ND	worldwide studies
Dussart, 1984	D/I	03/1976–04/1976	England/streams by a motorway
Dutka and others, 1998	D/I	ND	Burlington Ontario/highway-bridge runoff
Ellis and others, 1997	R/S	ND	England/receiving waters
Gish and Christensen, 1973	D/I	1970	Maryland and Virginia/highways, small streams and ponds (most near highways)
Gjessing and others, 1984	D/I	1981	Oslo, Norway/2-lane highway (laboratory assessment)
Helmers, 1996	D/I	07/1992–08/1994	Germany/right-of-way of Autobahn A8

PGE, platinum group element; R/S, review/summary; VPD, vehicles per day; ft, foot; mg/L, milligrams per liter; ppm, parts per million; %, percent]

Reference	Chemical analyte(s)	Chemical analysis matrix	Seasonality
Adams-Kszos andothers, 1990	deicers, properties, metals	water	yes
Baekken,1994	whole-effluent toxicity	biota, water	ND
Bardman, 1997	NA	ND	ND
Birdsall and others, 1986	metals	biota, sediment	ND
Boxall and others, 1993	metals, organics	biota, sediment	ND
Boxall and Maltby, 1997	organics	sediment	yes
Cline and others, 1982	total suspended solids	water	ND
Cooper and others, 1996	metals, organics	biota, water	ND
Corbett and Manner, 1975	deicers, majors, metals	water	yes (sodium chloride effects on water quality)
Crowther and Hynes, 1977	deicers	water	yes
Davis and George, 1987	oxygen demand, metals	biota, water	ND
Dickman and Gochnauer, 1978	deicers	water	ND
Dupuis, Kaster, and others, 1985	deicers, metals, organic carbon	biota, sediment, water	yes
Dupuis and others, 1999	deicers, metals, organics	biota, sediment, water (dissolved and whole)	ND
Dussart, 1984	NA (several mentioned but never tested)	biota	yes (“study took place at the end of the winter, so the maximum possible impacts of events, such as road salt, would be identifiable.” No comparison study was done in the summer.)
Dutka and others, 1998	whole-effluent toxicity	water	ND
Ellis and others, 1997	deicers, nutrients, metals, solids, organics, herbicides	biota, sediment, soil, water	deicers
Gish and Christensen, 1973	metals	biota, soil	ND
Gjessing and others, 1984	organics, whole-effluent toxicity	suspended solids, water (filtered)	One November snowmelt event included
Helmers, 1996	metals	biota	ND

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water—*Continued*

Reference	Report type	Dates of data collection	Location/ environmental setting
Horner and Mar, 1985	R/S	1977–82	Washington State/various highways
Karouna-Renier and Sparling, 1997	D/I	06/1994	Maryland/retention ponds collecting stormwater from various land uses including 3 highway sites
Kobriger and others, 1984	R/S	NA	ND/wetlands
Kramme and Brosnan, 1985	D/I	07/1984–11/1984	Ohio/rural highway, grassy right-of way (I-71), semirural highway (I-90), rural, 2-lane road
Madigosky and others, 1991	D/I	05/1989–06/1989	Louisiana/Roadside ditches in rural areas
Maltby, Boxall, and others, 1995	D/I	ND	England/stream near M1 motorway
Maltby, Forrow, and others, 1995	D/I	10/1990–07/1991, 04/1993	England/streams crossed by M1 motorway
McFarland and O’Reilly, 1992	R/S	ND	ND/various field and laboratory studies
McHardy and George, 1985	D/I	01/1977–05/1979	England/river on rural-to- urban gradient with sewage, urban, and highway inputs
McNeill and Olley, 1998	D/I	03/1995–05/1996	Southwest Scotland, highway outfalls on small rural streams crossed by the A74 motorway
Moore and Butler, 1994	R/S	ND	ND
Morgan and others, 1984	D/I	02/1981–12/1982	Vermont/lake surrounded by forest and limited residential land use
Mudre, 1985	D/I	1981–83	Virginia/6 streams with sites upstream, at the bridge, and downstream of a new highway (I-295)
Ney and van Hassel, 1983	D/I	04/1978–02/1979	Virginia/downstream of highway bridge
Portele and others, 1982	D/I	1980–81	Seattle, Washington/highways
Reifenhauser and others, 1995	P/A	ND	Germany/highways and roadways
Schafer and others, 1998	D/I	ND	Germany/plants grown in greenhouse in soils collected from roadside
Shively and others, 1986	D/I	01/1984–08/1985	Virginia/stream near highway, as well as urban area
Smith and Kaster, 1983	D/I	06/1980–06/1981	South Wisconsin/State Route-15, rural stream
Smith, 1976	R/S	ND	world-wide/roadside ecosystems (within 300 ft of the roadway)
Thrasher, 1983	Policy	NA	Continental United States/wetlands
van Hassel and others, 1980	D/I	04, 07, 11/1978 and 02/1979	Southwest Virginia/montain region

Reference	Chemical analyte(s)	Chemical analysis matrix	Seasonality
Horner and Mar, 1985	organics, oxygen demand, metals	water	ND
Karouna-Renier and Sparling, 1997	majors, metals, organics and properties	sediment, pond water	ND
Kobriger and others, 1984	deicers, metals, nutrients, organics, pesticides	biota, sediment, water	deicers
Kramme and Brosnan, 1985	herbicides, organics, properties	water (whole)	ND
Madigosky and others, 1991	metals	tissue	ND
Maltby, Boxall, and others, 1995	metals, organics	biota, sediment	ND
Maltby, Forrow, and others, 1995	metals, organics	bed sediment, runoff	Yes
McFarland and O’Reilly, 1992	deicers, oxygen demand, metals	soil, water	ND
McHardy and George, 1985	metals	tissue, water	ND
McNeill and Olley, 1998	deicers, metals, organics, sediment	water	deicers
Moore and Butler, 1994	metals, nutrients, organics, total suspended solids	water	ND
Morgan and others, 1984	deicers, majors, metals, nutrients	atmospheric deposition, sediment cores, water	yes (limited)
Mudre, 1985	metals	biota, sediment	ND
Ney and van Hassel, 1983	metals, properties	biota	ND
Portele and others, 1982	oxygen demand, metals, nutrients	biota, street sweepings, water	ND
Reifenhauser and others, 1995	metals	biota	ND
Schafer and others, 1998	metals	biota (plants), soil	NA
Shively and others, 1986	deicers, metals, nutrients, organics, pesticides	sediment, water	yes (discussed per parameter)
Smith and Kaster, 1983	deicers, metals, properties	biota, sediment, water	yes
Smith, 1976	metals	atmosphere, biota, soil	ND
Thrasher, 1983	NA	NA	ND
van Hassel and others, 1980	metals	biota, sediment, water	yes

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water—*Continued*

Reference	Report type	Dates of data collection	Location/ environmental setting
Wanielista and others, 1982	D/I	05/1981–05/1982	Central Florida/bridges over streams
Winters and Gidley, 1980	D/I	1976–77	California/roadways over streams and cut-slope runof

Reference	Biota studied	Method	Document method
Adams-Kszos and others, 1990	fish	toxicity testing	yes
Baekken,1994	benthic invertebrates, bivalves, fish	community assessment, tissue analysis	yes
Bardman, 1997	land (terrestrial) species	community assessments	ND
Birdsall and others, 1986	tadpoles	tissue analysis	yes
Boxall and others, 1993	benthic invertebrates	toxicity testing (using sediment and sediment extracts)	ND
Boxall and Maltby, 1997	benthic invertebrates	toxicity testing	yes
Cline and others, 1982	benthic algae, lichen, benthic invertebrates, moss	community assessment	yes
Cooper and others, 1996	benthic invertebrates, fish	tissue analysis	yes
Corbett and Manner, 1975	benthic fauna and vegetation	community assessment	ND
Crowther and Hynes, 1977	benthic invertebrates	toxicity testing, field and lab assessment of drift	yes
Davis and George, 1987	benthic invertebrates, plant tissue	community assessment, tissue analysis	yes

Reference	Chemical analyte(s)	Chemical analysis matrix	Seasonality
Wanielista and others, 1982	deicers, majors, metals, nutrients, organics	biota, soil, water	ND
Winters and Gidley, 1980	deicers, majors, metals, nutrients, organics	water	study took place in the winters of 1976 and 1977

Reference	Quality assurance	Comments
Adams-Kszos and others, 1990	reagent control	Found that a 50% dilution of bridge runoff was toxic to sunfish, especially for winter samples with salt concentrations greater than 11,000 mg/L.
Baekken,1994	controls, replicates	Tissue analysis and community assessment indicated chronic effects adjacent to highway runoff outfall.
Bardman, 1997	ND	Described methods to assess potential effects of bisecting habitats with transportation corridors.
Birdsall and others, 1986	replicates	Lead concentration in frogs is correlated with traffic density and sediment concentrations. Concentrations in frogs are high enough to affect predators.
Boxall and others, 1993	controls	Sediments from a site down gradient of a highway are toxic to the benthic invertebrate *Gammarus pulex*.
Boxall and Maltby, 1997	replicates	PAHs are the major toxins in sediment extracts from sites affected by highway runoff. Did spikes to determine that pyrene accounted for approx. 45% of total toxicity (fluoranthene, 16%; phenanthrene, 3.5%)
Cline and others, 1982	reference sites, replicates	Benthic communities and microinvertebrates were affected by sediment from construction, reducing the density, abundance and diversity, but high flows in this high gradient stream removed sediment deposits and therefore, limited damage spatially and temporally.
Cooper and others, 1996	replicates, clean containers, container blanks, field and trip blanks, field reagent spike	The water quality of the stream, and analysis of tissues indicated that the ecosystem was not affected by runoff from repaved asphalt/concrete pavement surfaces.
Corbett and Manner, 1975	control site	Sensitive species of benthic fauna or vegetation were not found at the highway site.
Crowther and Hynes, 1977	controls, replicates	Chloride pulses greater than or equal to 1,000 mg/L seemed to be the threshold to cause increased drift of species in the field. Static concentrations of 2,500 mg/L produced about 20% mortality in the laboratory.
Davis and George, 1987	replicates	Analysis of plant and animal tissue related to the chemistry of river water and subtle enough to relate to low-level secondary metal impacts.

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water—*Continued*

Reference	Biota studied	Method	Document method
Dickman and Gochnauer, 1978	algae, bacteria, other microbiota	community assessment on artificial substrate	yes
Dupuis, Kaster, and others, 1985	algae, fish, benthic invertebrates	community assessment, field toxicity, lab toxicity, tissue analysis, drift	extensive
Dupuis and others, 1999	aquatic invertebrates, fish, plants	community assessment, field toxicity, lab toxicity, tissue analysis	ND
Dussart, 1984	algae	community assessment	yes
Dutka and others, 1998	assorted species	a battery of toxicity tests, full strength and concentrated runoff	ND
Ellis and others, 1997	aquatic invertebrates	community assessment, tissue analysis	ND
Gish and Christensen, 1973	earthworms	tissue analysis	yes
Gjessing and others, 1984	algae, heterotrophic microorganisms (bacteria, fungi, protozoa), fish	acute toxicity tests (full strength and several dilutions)	yes
Helmers, 1996	plants	tissue analysis	yes
Horner and Mar, 1985	NA	compare estimated concentrations to regulated standards	ND
Karouna-Renier and Sparling, 1997	aquatic invertebrates	toxicity test	yes
Kobriger and others, 1984	numerous wetland species	NA	NA

Reference	Quality assurance	Comments
Dickman and Gochnauer, 1978	control sites, replicates, standard methods, intermethod comparison	Did controlled experiment in an unpolluted stream. Introduced pure sodium chloride at 1,000 ppm for 4 weeks. Salt reduced abundance and diversity of algae. Bacterial density was enhanced by suppression of predator organisms.
Dupuis, Kaster, and others, 1985	experimental controls, dry and wet weather sampling, replicates, compared	Did not demonstrate acute toxicity of highway runoff. Did observe some effects but not conclusively. Observed effects limited to outfall locations.
Dupuis and others, 1999	ND	Questions traditional long-term continuous exposure methods for toxicity tests. Notes that site specific criteria (based on local geochemistry) may improve evaluation of water-quality criteria. Noted that several of the reports reviewed did not contain sufficient documentation to evaluate results.
Dussart, 1984	replicates	Motorway runoff can affect algal flora, but further analysis is needed to identify the form of the effect.
Dutka and others, 1998	replicates, standard methods	Tested 9 bioassay methods using samples from the beginning and end of different storms. Found different results for different tests within and between storms. Did not detect acute toxicity, but did note some chronic effects (genotoxicity).
Ellis and others, 1997	ND	Referenced several British studies where highway runoff from sites with an ADT greater than 25,000 VPD increased trace-metal and organic chemical body burdens in aquatic invertebrates and reduced species diversity near highway outfalls. Reported immediate acute affects for chemical spills, but delayed chronic effects from instream contaminated sediment.
Gish and Christensen, 1973	controls, replicates	Detected elevated concentrations of metals in worms and soil near highways. Concentrations decreased with distance from the road edge, and decreasing traffic volume. Determined that there was bioconcentration from soil. Concentrations of lead in worms were toxic to predators in food chain.
Gjessing and others, 1984	controls, replicates	Used runoff from a two-lane highway on several species. Toxicity tests using fish eggs were done with highway sediments in a tank of slowly circulating tap water (may be questionable). None of the tests with runoff or sediments demonstrated acute toxicity.
Helmers, 1996	calibration, standards, blanks, replicates	Found aluminum, cerium, lanthanum, neodymium, and zirconium in grass tissues, as well as platinum that was linked to catalytic-converter emissions of vehicles. Over a few years, concentrations were increasing with increasing catalytic-converter use. Antimony and tin were also increasing, but attributable to asbestos-free brake linings.
Horner and Mar, 1985	ND	Method for estimating effects based on regulatory limits and a data base of highway-runoff water-quality values.
Karouna-Renier and Sparling, 1997	controls, replicates, blanks, spikes	Found that pond bottom sediments were not toxic, but test conditions minimized formation of bioavailable forms of constituents in the test chambers.
Kobriger and others, 1984	ND	Feasibility to use wetlands to treat highway runoff.

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water—*Continued*

Reference	Biota studied	Method	Document method
Kramme and Brosnan, 1985	benthic invertebrates, plants	bioassay/toxicity testing	yes
Madigosky and others, 1991	invertebrates	tissue analysis	yes
Maltby, Boxall, and others, 1995	benthic invertebrates	toxicity identification, evaluation, tissue analysis, lab toxicity tests	yes
Maltby, Forrow, and others, 1995	54 benthic invertebrate taxa, 29 species of aquatic fungi, 20 algal genera	community assessment	yes
McFarland and O'Reilly, 1992	algae, fish, plants	toxicity testing	ND
McHardy and George, 1985	algae	tissue analysis	yes
McNeill and Olley, 1998	benthic invertebrates	community assessment	yes
Moore and Butler, 1994	none	NR	ND
Morgan and others, 1984	algae, fish, phytoplankton, aquatic vegetation	community assessment	yes, some aspects of program limited
Mudre, 1985	benthic invertebrates, fish	community assessment, tissue analysis	yes

Reference	Quality assurance	Comments
Kramme and Brosnan, 1985	blanks, control treatment areas in close proximity experiment controls, standards, spikes	Monitored water-quality impacts of the herbicides 2,4-D and Picloram, and PAHs from road seal-coating material. Each chemical was tested at one urban roadway site. Found that concentrations in runoff were low or undetectable. Neither pesticide affected plant germination rates but the 2,4-D in the runoff from the first postapplication storm did show some chronic effect in growth. Tests with the PAHs, however, did not indicate lethality for the microinvertebrates *Daphnia magna*.
Madigosky and others, 1991	control site	Noted significant increases in the concentrations of lead cadmium and aluminum in the tissues of crayfish collected from roadside ditches in Louisiana when compared to a control site. The elevated concentrations did not seem to affect the crayfish, but were high enough to be a helth hazard to other organisms that use crayfish as a food source.
Maltby, Boxall, and others, 1995	replicates	Stream water mixed with runoff was not toxic but experiments show that aromatic hydrocarbons are accumulated by biota in direct proportion to exposure concentrations. Identified PAHs as the primary toxicant, therefore, population differences among sites in the field studies could be caused by organism's response to sublethal effects.
Maltby, Forrow, and others, 1995	replicates, standards	Demonstrated differences in sediment- and water-quality from upstream to downstream and resultant loss of pollution-sensitive taxa of macroinvertebrates and difference in relative abundance of others. Hydraulics and sediment deposition caused by potential highway runoff may affect communities.
McFarland and O'Reilly, 1992	ND	Did not find calcium magnesium acetate (CMA) to be particularly toxic.
McHardy and George, 1985	control site, replicates	In comparison to stream water, algae accumulated metals at all sites with significant positive correlations for cadmium and zinc. Metal concentrations generally increased along the rural to urban gradient with elevated metals near outfalls of sewage treatment plants, as well as highways, and other nonpoint sources.
McNeill and Olley, 1998	control sites	At many sites, "normal" highway runoff did not have a substantial effect on downstream biota along this new highway with grassy swale BMPs. However, problems with fine sediment and small routine spills from auto accidents were noted as problems.
Moore and Butler, 1994	ND	There are limited data describing the effects of highway runoff on receiving water bodies. Additionally, there is a lack of data describing water quality and the resultant aquatic impacts.
Morgan and others, 1984	ND	Phosphorus loading in a lake is controlled by sedimentation and internal loading. The construction of I-91 in the watershed has caused increased sulfate loading which has promoted internal phosphorus recycling.
Mudre, 1985	control sites, replicates, spikes	Detected elevated concentrations in tissues and some shifts in community structures at sites along a relatively low-volume highway (ADT: 6,000–15,000 VPD). Found substantial site-to-site variability that was caused by interrelated physical and chemical characteristics of these otherwise pristine stream sites.

Table 2. Documented metadata for reports examining the effects of highway runoff on biota in receiving water—*Continued*

Reference	Biota studied	Method	Document method
Ney and van Hassel, 1983	fish (6 species)	tissue analysis	yes
Portele and others, 1982	algae, fish, zooplankton	bioassays/toxicity	yes
Reifenhauser and others, 1995	plants	tissue analysis	yes, but not detailed
Schafer and others, 1998	plants	tissue analysis	yes
Shively and others, 1986	benthics (many species and taxa)	community assessment	yes (water and sediment), no (benthic assessment)
Smith and Kaster, 1983	benthic invertebrates	community assessment	yes
Smith, 1976	insects, plants, worms	tissue analysis	ND
Thrasher, 1983	ND	“ecological impact assessment”	ND
van Hassel and others, 1980	benthic invertebrates, fish	tissue analysis	yes
Wanielista and others, 1982	algae, plants	plant tissue analysis, algal bioassay/toxicity	yes
Winters and Gidley, 1980	algae	bioassay/toxicity testing (in lab)	yes

Reference	Quality assurance	Comments
Ney and van Hassel, 1983	replicates, spikes	Found that fish species associated with sediments had higher metal contents than water-column species, and that different tissues had different affinities for different metals.
Portele and others, 1982	clean containers, field and trip blanks	Soluble fraction of highway runoff adversely affected algae and zooplankton. Suspended solids caused high mortality of rainbow trout.
Reifenhauser and others, 1995	ND	Examined use of grass cultures for localized heavy-metal deposition rates.
Schafer and others, 1998	agricultural-soil controls	Plants in soil affected by highway runoff concentrate heavy metals and PGE.
Shively and others, 1986	yes (no details)	No significant effects on benthos were observed at either site. No significant effects in water-quality parameters were investigated (except iron and lead).
Smith and Kaster, 1983	replicates, control site	Monitored effects of a low-volume highway (ADT —7,000–8,000 VPD). Effects of highway runoff were not distinguishable from effects of habitat characteristics at each site. Need to monitor and document physical stream characteristics, especially substratum and stream current. Differences in measured biological parameters controlled by site characteristics.
Smith, 1976	ND	Lead in particulate form. Found that concentrations decrease with distance from the road. Lead concentrations increase with traffic volume. Particulate load goes out exhaust into atmosphere and is dispersed into the roadside environment.
Thrasher, 1983	NA	Discusses alternatives to construction on wetlands, avoidance, study, mitigation, and reconstruction.
van Hassel and others, 1980	replicates	Tissue concentrations of source metals increased with increasing traffic density. Stream water was not highly contaminated but sediments were. (Sites ranged from an ADT of 50–15,000 VPD).
Wanielista and others, 1982	replicates, standards, reagent controls	Lead levels were much higher in plants collected at bridge sites than control sites. Bridge areas were dominated by fewer species than controls. Stormwater runoff can become toxic to receiving water bodies when mixed in high concentrations.
Winters and Gidley, 1980	replicates	Heavy metals inhibited growth. Elevated levels of nutrients stimulated growth.

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Buckler and Granato—ASSESSING BIOLOGICAL EFFECTS FROM HIGHWAY-RUNOFF CONSTITUENTS—OFR 99-240