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Standard Guide for Fish and Wildlife Incident Monitoring and Reporting[1]

This standard is issued under the fixed designation E 1849; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 State and Federal agencies as well as industry have requested guidance for collecting, reporting, and interpreting fish and wildlife incident data. This guide covers planning and completing a thorough investigation to determine an incident's cause. Recommendations for documenting and reporting incidents are provided. A summary of the information necessary for completing a risk assessment and information on how fish and wildlife incident data are considered for regulatory decisions are also provided.

1.2 Several agencies collect information and maintain databases regarding fish and wildlife incidents. A list of these databases and the types of information they contain are included. Agencies with regulatory authority relating to fish and wildlife incidents are listed and laws pertaining to fish and wildlife incidents are summarized.

1.3 For the purposes of this guide, a fish or wildlife incident is defined as an allegation of an adverse effect on nontarget fish and wildlife species. By today's detecting standards, adverse effects data are usually limited to mortalities. However, as biological monitoring improves, sublethal effects data may be more readily quantifiable and reported.

1.4 This guide is arranged as follows:

1.5 The values stated in both inch-pound and SI units are to be regarded separately as the standard. The values given in parentheses are for information only.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 5.

2. Referenced Documents

2.1 *ASTM Standards:*

D 4687 Guide for General Planning of Waste Sampling[2]

E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians[3]

E 943 Terminology Relating to Biological Effects and Environmental Fate[3]

E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses[3]

E 1192 Guide for Conducting Acute Toxicity Test on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians[3]

E 1241 Guide for Conducting Early Life-Stage Toxicity Test with Fishes[3]

E 1295 Guide for Conducting Three Broad, Renewal Toxicity Tests with Ceriodaphnia Dubia[3]

E 1367 Guide for Conducting 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods[3]

E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing[3]

E 1463 Guide for Conducting Static and Flow-Through Acute Toxicity Tests with Mysids from the West Coast of the United States[3]

E 1525 Guide for Designing Biological Tests with Sediments[3]

E 1705 Terminology Relating to Biotechnology[3]

[1] This guide is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fateand is the direct responsibility of Subcommittee E47.01on Aquatic Assessment and Toxicology.

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[2] *Annual Book of ASTM Standards*, Vol 11.04.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

3. Terminology

3.1 *Definitions of Terms Specific to This Standard*—The words "must", "should", "may", "can", and "might" have very specific meanings in this guide. "Must" is used to express an absolute requirement, that is, to state that the action ought to be designed to satisfy the specified condition, unless the purpose of the action requires a different design. "Must" is only used in connection with factors that directly relate to the acceptability of the action. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "is desirable," "is often desirable," and "might be desirable" are used in connection with less important factors. "May" is used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

4. Significance and Use

4.1 *Laws and Regulations*—In the United States there are federal laws that either directly or indirectly suggest the use of fish and wildlife incidents in the ecological risk assessment process. These laws are: Federal Insecticide, Fungicide, and Rodenticide Act; Endangered Species Act; Resource Conservation and Recovery Act; Toxic Substances Control Act; Clean Water Act; Comprehensive Environmental Response, Compensation, and Liability Act; and the Migratory Bird Treaty Act. Additionally, many states have their own laws relating to fish and wildlife incidents. This guide provides general guidance for monitoring and reporting fish and wildlife incidents and does not relieve the user of additional requirements found in specific regulations.

4.2 *Benefits of Maintaining Records*:

4.2.1 Incident reports are instrumental in identifying or confirming ecological risks associated with a particular contaminant. Incident reports may also help identify species particularly sensitive to certain chemicals, trends in chemicals, crops, and fish and wildlife, and pinpoint geographic areas impacted by contaminants.

4.2.2 Incident data have formed the basis for the regulation of some pesticides and solid waste in the past. Instances where incident data have affected pesticide and solid waste regulation include: severe restriction of the use of tributyltin, a marine antifoulant, due to reported adverse effects on Pacific oyster growth; cancellation of diazinon's use on golf courses and sod farms due to reported bird mortalities; voluntary cancellation of carbofuran use on corn due to reported bird mortalities; and restriction of the use of carbofuran on grapes and rice and azinphos-methyl on sugarcane due to reported bird or fish mortalities.

4.2.3 Incident data have been useful to the Office of Solid Waste of the U.S. Environmental Protection Agency (EPA) in the regulation of cyanide. Hundreds of bird kills have been caused by the use of cyanide in the leaching of gold. As a result, gold mining companies have been developing improved leaching technology to reduce exposure to fish and wildlife species.

4.3 *Ecological Incident Databases*:

4.3.1 There are many databases that contain information on fish and wildlife incidents. The reliability of these databases may benefit from the recommendations on collection, investigation, reporting, and interpretation techniques contained in this guide. An outline of the databases as they currently exist or will exist in the very near future follows:

4.3.2 *Ecological Incident Information System (EIIS)*—Maintained by the EPA's Office of Pesticide Programs, the EIIS is a DBASE III Plus[4] software package designed as an application tool for state and federal agencies to enter and submit incident data relating to pesticides. The software was distributed to 175 state and federal agencies and industry in 1993. The database includes information for location of incident, species affected, magnitude of effect, pesticide(s) and formulation, application rate and method, and circumstances under which the incident occurred. The data are searchable by all parameters considered in ecological risk assessments by the EPA.

4.3.3 *Fish Kills in Coastal Waters 1980–1989*—In 1991, the National Oceanic and Atmospheric Administration conducted a survey of fish kills and their causes along all United States coastlines. The report summarizes results from across the Nation to identify, report, and assess the causes of fish kills in coastal rivers, streams, and estuarine waters between 1980 and 1989. The location, extent, severity, timing, and cause of over 3600 fish-kill events are documented. These data are also available in database format.

4.3.4 *Epizootic and Diagnostic Databases*—The Department of Interior National Biological Service (NBS) maintains two databases recording avian mortalities. The first, the epizootic database, includes data gathered on field investigations by the National Wildlife Health Center (NWHC) staff, diagnostic evaluation and laboratory testing done within NWHC, as well as verifiable reports of mortality, diagnostic evaluation, or laboratory results from other agencies. The second, the diagnostic database, contains information from the NWHC necropsy and laboratory results. The information from both databases represents what is observed and reported to NWHC and subsequent field investigations or diagnostic evaluations made on a subsample of reported events.

4.3.5 *Southeastern Cooperative Wildlife Disease Study (SCWDS)*—The SCWDS maintains a database containing information regarding wildlife mortalities in the 14 member states (AL, AR, FL, GA, KY, LA, MD, MO, MS, NC, SC, TN, VA, WV) and Puerto Rico. Available information includes clinical history, location and extent of mortality, species involved, postmortem findings, and results of tests for toxins and microbes. The data are searchable by species, diagnosis, date, and location. Information contained in the database is not intended for citation in the scientific literature. The SCWDS should be contacted if citable information is needed.

4.3.6 *Individual State Agencies*—A total of 102 state agencies collect fish and wildlife incident data associated with

[4] A registered trademark of Borland International Inc., 100 Borland Way, Scotts Valley, CA 95066.

pesticide poisoning. The level of reporting varies considerably among state agencies. A limited number of state agencies store the information in a database. Most of the agencies store the data as hard copy.

4.3.7 *The Wildlife Incident Investigation Scheme*—The Central Science Laboratory, Ministry of Agriculture, Fisheries and Food (MAFF) of the United Kingdom has been recording incident data related to vertebrate mortality in Great Britain since 1964. Bee mortality in Great Britain has been recorded since 1981. Over the years this scheme has widened its scope and is now able to detect animal poisonings caused by most pesticides. The majority of the incidents that are reported include vertebrate wildlife, companion animals, and beneficial insects. Reports are published annually.

4.3.8 *Canadian Cooperative Wildlife Health Centre*—This is Canada's national organization for wildlife health services and surveillance. It was established in 1992 and is supported by federal and all provincial and territorial governments, as well as by nongovernment sources. Regional centres throughout Canada provide services such as disease diagnosis and regional consultation and provide data on wildlife disease occurrences to a national database. Data come from provincial veterinary diagnostic laboratories as well as from the regional centres themselves. The national database uses the Paradox[4] relational database software. Data are entered in a total of 54 fields. Hierarchical codes are used for species and diagnoses. Diagnoses are recorded by anatomical, pathological, and etiological (causal) criteria using the system created for the Ontario Ministry of Agriculture, Food, and Rural Affairs. The data are searchable by any field. This database and the diagnostic examinations from which the data are derived constitute the wildlife disease surveillance mechanism of the CCWHC. Surveillance is passive with respect to acquisition of specimens, and detection of mortality or morbidity is done by professional wildlife personnel and the public.

4.4 *Ecological Risk Assessments*:

4.4.1 Reported fish and wildlife incidents are used in ecological risk assessments by the U.S. Environmental Protection Agency and other Federal and State agencies, such as the U.S. Fish and Wildlife Service and the California Department of Fish and Game.

4.4.2 *Risk Assessments Related to Pesticides*—In order to understand the effects of pesticides in the environment, the Ecological Effects Branch (EEB), within the Office of Pesticide Programs (OPP) of the EPA conducts risk assessments to determine the effects of pesticides on nontarget fishes, mammals, birds, invertebrate, and plant species. In order to complete a risk assessment, the EPA must review toxicity and environmental fate data. Toxicity data include acute and chronic laboratory data for freshwater and marine organisms and terrestrial wildlife. Environmental fate data include photolysis, hydrolysis, solubility, and field dissipation data **(1)**.[5] Field study and ecological incident data are also considered in completing the risk assessment **(2)**. According to the Ecological Fate and Effects Task Force, aquatic and terrestrial field studies are no longer required, except in unusual circumstances. The decisions will now be made based on laboratory testing, incident data, and other information which can easily be collected to enable the program to better characterize risk.

4.4.2.1 These data are evaluated and used in a "weight of the evidence" approach to assess risk. For example, if the use of the pesticide is expected to exceed established OPP risk criteria for the protection of nontarget species, based on laboratory data, then available field data (both field studies and incident data) are evaluated. Reported incidents confirm risk that has been previously identified by laboratory testing or identify risk that has not been predicted by evaluating the laboratory data.

4.4.2.2 There are several aspects of incident data that are considered when evaluating a pesticide during an ecological risk assessment. These include species affected, number of individuals of each species affected, the number of incidents reported, location of the incidents, pesticide use site, and circumstances under which the incident occurred. There are several examples where EPA has evaluated the data and determined that while a particular use of a chemical required cancellation, other uses of the same chemical did not require cancellation. Alternately, pesticide application methods might be modified to successfully reduce risk to an acceptable level.

5. Precautionary Steps and Safety

5.1 Investigators initiating a field investigation of a fish or wildlife mortality event should presume that the event is the result of an outbreak of contagious disease until proven otherwise. With this in mind, investigators must take every precaution to ensure that disease is not spread to humans, other wildlife, or domestic animals. Guidelines for ensuring containment of disease outbreaks are reviewed in Refs **(3)**, **(4)**, and **(5)**.

5.2 Many materials (for example, pesticides, hazardous wastes, solid wastes) can harm humans if inadequate precautions are taken. Therefore, skin contact with all unknown materials and solutions should be minimized by wearing appropriate protective clothing, especially when washing equipment, putting hands in unknown solutions or soil that might be contaminated, or handling contaminated plants or animals.

5.3 Information on toxicity of the material to humans **(6)**, recommended handling procedures **(7)**, and chemical and physical properties of the material, if known, should be studied before handling. Special procedures might be necessary with radio-labeled materials **(8)** and with materials that are, or are suspected of being, carcinogenic **(9)**. Guide D 4687 recommends protective safety equipment based on the level of hazard to human health. Also, special safety precautions may be appropriate to minimize risks from handling fish or wildlife.

5.4 Although disposal of unknown solutions, soils, and organisms poses no special problems in many cases, health and safety precautions and applicable regulations should be considered before beginning the site inspection and collecting organisms. Removal or degradation of suspected toxic material might be desirable before disposal of solutions, soils, and organisms. Hazardous materials must be disposed of in accordance with state and federal regulations.

[5] The boldface numbers given in parentheses refer to the list of references at the end of the text.

5.5 Individuals participating in on-site investigations can protect themselves and others by wearing the appropriate type of protective clothing (including coveralls, boots, or other protective footwear; respirator or face mask; and gloves) and by cleaning up and disinfecting themselves, their clothing, and equipment before leaving the site (see Appendix X1). Washing your hands with soap and water is an excellent safeguard, in addition to decontaminating boots and equipment with disinfectant. Personnel should wear protective equipment when response activities involve known or suspected atmospheric contamination; when vapors, gases, or airborne particulates may be generated; or when direct contact with skin-affecting substances may occur. Respirators can protect lungs, gastrointestinal tract, and eyes against airborne toxicants. Chemical-resistant clothing can protect the skin from contact with skin-destructive and skin-absorbable chemicals. Good personal hygiene limits or prevents ingestion of material.

5.6 The safety of investigators and the public should always be a primary concern when fish and wildlife incident sites are investigated. This is especially true for kills that involve spills or drift of unknown or hazardous materials. The level of protection selected should be based primarily on the following: type(s) and measured concentration(s) of chemical substance(s) in the ambient atmosphere and its toxicity and potential or measured exposure to substances in air, splashes of liquids, or other direct contact with material due to work being performed. In situations where the type(s) of chemical(s), concentration(s), and possibilities of contact are not known, the appropriate level of protection must be selected based on professional experience and judgement until the hazards can be better characterized. Guide D 4687 uses four levels to classify hazards to human health. Each level has specific protective (safety) equipment required for dealing with the potential dangers associated with sampling and analyzing a waste:

5.6.1 *Level A*—Should be worn when the highest level of respiratory, skin, and eye protection is needed.

5.6.2 *Level B*—Should be selected when the highest level of respiratory protection is needed, but a lesser level of skin protection. Level B protection is the minimum level recommended on initial site entries until the hazards have been further defined by on-site studies and appropriate personnel protection utilized;

5.6.3 *Level C*—Should be selected when the type(s) of airborne substance(s) is (are) known, the concentration(s) is (are) measured, and the criteria for using air-purifying respirators are met; and

5.6.4 *Level D*—Should not be worn on any site with respiratory or skin hazards (see Appendix X1).

6. Determining the Cause of the Incident

6.1 In many cases, circumstances surrounding a contaminant-related die-off incident and symptoms of affected wildlife are difficult to distinguish from circumstances and signs caused by biological or physical and natural agents. For this reason, investigators should treat each incident as potentially infectious. Refer to Friend **(4)** and Davidson and Nettles **(3)** for a thorough review of wildlife diseases and their clinical symptoms. Some diseases that may resemble wildlife die-offs caused by pesticides or other contaminants include botulism, salmonella, trichomoniasis, and duck virus enteritis. Refer to Meyer and Barclay **(5)** for a thorough review of fish kills caused by diseases and contaminants. Investigators should rely on fish and wildlife disease specialists to obtain a definitive diagnosis of the disease agent.

6.1.1 Investigations of incidents suspected to have originated from a toxic substance must proceed as though the cause is unknown. All factors must be checked or eliminated unless there is firm evidence that specific causes are not involved. The investigation should proceed through a process of elimination.

6.2 *Common Causes of Fish Mortality*:

6.2.1 *Chemical Agents*:

6.2.1.1 Fish kills caused by toxic substances have been enumerated and assessed by the EPA and National Marine Fisheries Service (NMFS). Common causative agents include acids from industrial discharges, trace-metals copper, zinc, cadmium, lead, and mercury from mining activity, trinitrophenols from industrial discharges, ammonia and nitrate from fertilizer and sewage, detergents from industrial and municipal discharges, untreated (raw) and chlorinated sewage, cyanide from mining activity, and organophosphate and carbamate insecticides and other pesticides from agricultural drift and runoff. Some chemicals kill both plants and animals while other compounds may affect only plants, only animals, or only certain species or sizes of animals. Species of fish and other aquatic life vary in their susceptibility to toxic substances. When a kill occurs, it is important to determine if other organisms, such as algae, zooplankton, sandworms, snails, insects, crabs, bivalves, crayfish, frogs, turtles, or snakes, are still alive.

6.2.1.2 There are a number of sources of toxicity information for aquatic life. One of the most complete sources of aquatic toxicity information is the database AQUIRE, maintained by the Office of Toxic Substances of EPA. Another source of toxicity data of pesticides is the Ecotoxicity Database maintained by the Office of Pesticide Programs. Other sources include U.S. Environmental Protection Agency,[6] American Fisheries Society **(10)**, U.S. Fish and Wildlife Service **(11)**, Mayer **(12)**, and Weed Science Society of America **(13)**.

6.2.1.3 Frequently, the introduction of a toxic substance causes no change in water chemistry, but may leave residues in the water, sediment, or animal tissues. These media should be measured for residues of toxic chemicals because the results may yield significant information about exposure. Residue analyses may provide evidence that a suspect chemical has been detected in water at quantities known to be toxic, significant differences exist in the chemical composition of waters between the kill and the reference sites, a suspect chemical is present in the sediment at the kill site, or residues of a suspect chemical are in gill or other tissues of fish from the incident site. Other tests which may assist in the investigation include toxicity tests to determine whether water from the incident site is toxic as compared to a reference site and

[6] The U.S. EPA has published water quality criteria documents for many common pollutants. Documents are available for many pesticides, heavy metals, bacteria, ammonia, and water quality characteristics, such as pH and hardness. These are available through the National Technical Information Service, 5285 Port Royal Road, Springfield VA 22161.

laboratory assays of acetylcholinesterase or ATPase enzyme activity in fish to demonstrate differences between the kill and reference sites.

6.2.1.4 A complete discussion of common chemical agents responsible for fish kills is presented in Appendix X2.

6.2.2 *Biological Agents*:

6.2.2.1 Much of the information for this section was taken from, and for a more complete description see Chapter 6 of Ref **(5)**.

6.2.2.2 Outbreaks of disease are seldom the result of a single factor. Three factors are involved in every potential disease situation: susceptible hosts, pathogenic organisms, and predisposing environmental conditions. All must be present when an epizootic occurs. Snieszko **(14)** listed decreased immunological response, poor genetic resistance, temperature stresses, pollution, unfavorable water chemistry, and other adverse conditions as some of the possible predisposing factors. Adverse conditions may include factors such as crowding, inadequate food supply, spawning activity, storms, and seasonal changes.

6.2.2.3 Pathogens rarely overwhelm a healthy population of fish. Therefore, it is important to look for underlying factors that may have contributed to the occurrence. In fish kills caused by parasitic or infectious agents, there is a gradual buildup in the rate of loss as the weakest, most severely affected animals die first. A variety of infectious agents have been identified as the cause of fish kills in natural waters, among which viruses, bacteria, fungi, and parasitic organisms are prominent. Toxins from algae may also be responsible for fish kills.

6.2.2.4 It is often difficult to identify viral, bacterial, fungal, or parasitic agents in the field. Specific guidance for sample preservation should be obtained from the analytical facility. In general, the fish should be bagged or wrapped in plastic, packed with wet ice, and transported to the laboratory as soon as possible. The fish must not be frozen.

6.2.2.5 A complete discussion of common biological agents responsible for fish kills is presented in Appendix X2.

6.2.3 *Natural and Physical Agents*:

6.2.3.1 Much of the information for this section was taken from a U.S. Fish and Wildlife manual. For a more complete description see Chapter 5 of Ref **(5)**.

6.2.3.2 Fish kills occur as a direct result of natural causes. Causative agents that have been identified are oxygen depletion, gas supersaturation, turnovers, toxic gases, natural toxic substances, sudden or excessive temperature changes, lightning, and others. Usually there is sufficient evidence at the site to help the investigator accurately determine if the kill was due to a natural cause.

6.2.3.3 A complete discussion of physical and natural agents responsible for fish kills is presented in Appendix X2.

6.3 *Common Causes of Wildlife Mortality*—As with fish kills, causes of wildlife mortality can be very diverse. Causes of mortality include chemical exposure, biological agents, and physical injury and natural agents. Investigations of wildlife kills should proceed with the initial assumption that the cause is unknown.

6.3.1 *Chemical Agents*:

6.3.1.1 Sometimes, chemical exposure is obvious. Dying birds seen drinking irrigation runoff water from a field which was sprayed recently with an organophosphate insecticide have probably been poisoned. Ducks found dead in a containment pond from a cyanide heap leaching process probably died from exposure to cyanide. Sometimes, chemical exposure is not as obvious. A cormorant rookery suffers almost complete nesting failure the spring following a particularly severe winter. The failure is not due to the colony suddenly being exposed to an application of a pesticide but to exposure of the adults to various organic chemicals remobilized in the environment. This can result from severe scouring of nearby river sediments during heavy winter flows.

6.3.1.2 The risk of chemicals to animals is dependent on both toxicity and exposure. The toxicity of the insecticide carbofuran is extremely high, less than 1 mg/kg, while that of 2,4-D is considered low, 500 mg/kg. Exposure to toxic materials may occur by ingestion, inhalation, or dermal absorption. In birds, the most common method is by ingestion: eating or drinking something toxic. Birds sprayed directly or exposed to an aerosol suspension of a pesticide usually would be considered as functionally exposed by ingestion because they will preen the foreign substance off their feathers and ingest it. Birds can also absorb pesticides directly through their feet by perching on a substrate treated with a toxic material. This type of exposure has been demonstrated with raptors utilizing fruit and nut orchards during the winter following application of organophosphate dormant sprays. There have been only one or two cases where mortality due to inhalation of a toxicant by birds was documented.

6.3.1.3 For mammals, the most common means of exposure is ingestion. Nontarget animals occasionally consume rodent poisons set out for control of ground squirrels or other rodent species. Secondary poisoning of predatory or scavenging birds or mammals may occur if poisoned rodents are ingested. Burrow fumigants for ground squirrel control cause death by inhalation of toxic materials. The animals typically die underground from fumigants and are not observed in distress.

6.3.1.4 A complete discussion of chemical agents responsible for wildlife kills is presented in Appendix X2.

6.3.2 *Biological Agents*:

6.3.2.1 Certain algae and dinoflagellates can release toxins that will kill birds or mammals if they consume water containing these toxins. Because of the wide range of diseases which can affect wildlife, and the great differences in susceptibility to a specific disease between species, a detailed discussion of diseases is best dealt with in other texts dedicated to that topic **(3,4,15)**. Because of the complexity of many viral, bacterial, and parasitic diseases and the fact that disease symptoms can often mimic symptoms characteristic of exposure to pesticides or other toxins; the field investigator should try to work closely with a wildlife veterinarian whenever possible.

6.3.2.2 A discussion of biological agents responsible for wildlife kills is presented in Appendix X2.

6.3.3 *Physical and Natural Agents*:

6.3.3.1 Naturally occurring phenomena can be responsible for significant wildlife kills. Lightning has been identified in several incidents involving waterfowl. Sudden, unseasonal, changes in weather conditions, such as cold fronts can adversely effect populations **(16)** causing localized die-offs. Large hail stones are also known to have caused massive mortality of birds.[7] Migratory stress and adverse weather conditions or low food supplies during the time migratory herds or flocks arrive may result in large die-offs of weakened animals.

6.3.3.2 A discussion of physical and natural agents responsible for wildlife kills is presented in Appendix X2.

7. Investigation of Incidents

7.1 *Quality Control and Standard Operating Procedures*:

7.1.1 The materials and methods (for example, standard operating procedures) used in an investigation should be referenced in the final report along with any quality assurance procedures used in the analyses. Standard quality control procedures, data reduction, and reporting should be in compliance with accepted standards such as Refs **(17)** and **(18)**. Water, fish, invertebrate, and sediment samples should be collected and analyzed using accepted test methods such as those developed by the ASTM procedures. Acute and chronic toxicity tests should be performed using accepted standards such as EPA procedures **(19)** and ASTM procedures.[8] It is more important to reference the materials and methods used in the investigation than to use any particular method.

7.1.2 Use a recognized procedure if available or other scientifically valid procedure. Water quality analyses for major ionic constituents or compounds should be performed using accepted procedures such as ASTM test methods from Committee D-19 or Ref **(17)**. Results of water quality analyses should be reported in µg/L (ppb) for pesticides and metals, and in mg/L (ppm) for other major constituents. Results of sediment analyses should be reported as dry weight, in µg/g (ppm) for metals and ng/g (ppb) for pesticides and other organic compounds of concern. Reporting values relating precision and bias for the analyses greatly adds to the credibility of the information provided in the investigation; reporting values for replicate samples, controls, sample splits, and standard toxicant samples can be helpful in judging the validity of the data.

7.1.3 Precision should be assessed with each sample set for each analysis type. Precision should be expressed in terms of relative error as the percent deviation of the duplicate results from the original results obtained. The equation for determining precision is as follows:

$$RPD = [(D1 - D2)/[(D1 + D2)/2] \times 100 \quad (1)$$

where:
RPD = relative percent difference,
$D1$ = first sample results, and
$D2$ = second sample results.

7.1.4 Bias of the analysis should be assessed on a regular basis, with each set of samples for each analysis type, by comparing the analytical results of internal quality assurance samples with accepted concentrations. Bias should be expressed in terms of relative error as the percent deviation of the analytical results from the known values. Bias is calculated as follows:

$$Acc = [(Da - D)/D] \times 100 \quad (2)$$

where:
Acc = percent accuracy,
Da = analysis value of quality assurance sample, and
D = accepted value of quality assurance sample.

In the absence of internal quality assurance samples, bias can be assessed using recovery of spiked samples. Recovery is calculated as follows:

$$PSR = [(Ds - Du)/S] \times 100 \quad (3)$$

where:
PSR = percent spike recovery,
Ds = measured value of the spiked sample,
Du = measured value of the unspiked sample adjusted for the dilution of the spike, and
S = expected value of the spike contribution alone.

7.1.5 As part of the good quality assurance procedures, standard operating procedures (SOPs) should be developed for all procedures and tests conducted on samples in an investigation. The SOP's should be based on referenced and accepted procedures.

7.2 *Planning Field Investigation*:

7.2.1 Investigating a fish and wildlife kill requires keen observation and an inquisitive mind. Familiarity with literature on fish and wildlife kill investigations, and knowledge of the resources and operational and administrative procedures available are also invaluable. The possibility exists that questions of legal liability will result from an investigation and that a judge or jury may scrutinize the record of the investigation. There is a need for a carefully planned, properly conducted, and legally defensible investigation.

7.2.2 The written record for an investigation should include forms to record data, names and telephone numbers of persons to be contacted, names and telephone numbers of other departments of the organization to be contacted (for example, analytical facility, diagnostic laboratory, and law enforcement), maps of the incident area, sample logbook, and chain-of-custody material. Photographs of the general site of the incident (including an unique marker (for example, odd-shaped tree)) and the positions of the victims in relation to the site are valuable in enforcement cases.

7.2.3 Before entering an area, the hazard to personnel should be assessed, necessary safety equipment obtained and appropriate precautions be followed (see Appendix X1). A list of recommended supplies and equipment for an environmental sampling kit is found in Appendix X3. Routine maintenance is required to keep the needed equipment and supplies in ready condition. A maintenance check sheet should be kept and periodic checks should be made in accordance with the manufacturer's recommendations. This is especially important when battery-operated gear is to be used. If possible, have available a backup system of analysis that does not require batteries. Culture media and solutions must be regularly replaced to ensure that these products are always fresh and

[7] Fletcher, M., Personal Communication, Central Science Laboratory, Slough, Berks, Britain.

[8] For example, see Guides E 729, E 1192, E 1241, E 1295, E 1367, and E 1463 and Terminology E 1705.

ready to use. Special gear or chemicals may require specific storage conditions to prevent deterioration or contamination.

7.3 *Interagency Coordination*:

7.3.1 Coordinating a fish and wildlife incident investigation starts before the field investigation. A list of names, addresses, and telephone numbers of persons or agencies to be notified in the event of a fish or wildlife incident should be maintained by each natural resource agency. Once a fish or wildlife incident has been reported, the appropriate persons should be contacted as soon as possible. If more than one agency is involved in an investigation, all participants should be kept fully informed so that the investigation is thorough and effective. A number of flow charts have been published to help coordinate incident investigations **(20,21)**.

7.3.2 Usually, the state fish and wildlife agency should be the first point of contact when a fish or wildlife incident occurs. If an incident involves migratory fish or wildlife or federally designated threatened or endangered species, the U.S. Fish and Wildlife Service (USFWS) should be notified. Additionally, if the incident is caused by a pesticide, the incident should also be reported to the Office of Pesticide Programs of EPA. If, during the initial investigation, evidence exists that a fish or wildlife incident occurred as a result of criminal or negligent behavior, the on-site team should immediately contact the appropriate state enforcement agency. The documentation of potential witnesses, background information, and samples collected is invaluable in producing a strong case.

7.3.3 In a fish and wildlife incident investigation, one individual should be designated as the contact person for the news media. This helps avoid contradictory reports and confusion. Publicity and news releases during the entire period of the investigation should be limited to factual accounting of the conditions observed. Conjecture as to the probable cause of the mortality or the persons or company that might be responsible must be avoided. Information that might be released includes a description of the fish and wildlife mortality, the extent of mortality, when the kill was first observed, the duration of the kill, and the names of agencies involved in the investigation.

7.4 *Chain of Custody*:

7.4.1 In some cases, fish or wildlife die-off incidents may lead to legal proceedings against an individual(s) (that is, where a pesticide is illegally used to kill wildlife). Investigators should assume that any investigation has the potential to become a legal case. In any investigation, chain of custody documentation is required to demonstrate that evidence can be accounted for at all times.

7.4.2 The chain of custody is defined as the witnessed, written record of all individuals who have maintained unbroken control over (custody of) the evidence since its acquisition by an agency; several guidelines have been proposed **(22)**. The chain of custody begins when an item of evidence is collected and is maintained until final disposition. Each individual in the chain of custody is responsible for an item of evidence to include its care, safekeeping, and preservation while it is under her or his control. Chain of custody procedures require that possession of samples be traceable from the time the samples are collected until completion and submittal of analytical results. Generally, a sample is considered under custody if it is in actual possession, it is in view after in physical possession, or it is placed in a secure area (accessible by or under the scrutiny of authorized personnel only).

7.4.3 Because it is possible that any item or specimen acquired during an investigation may have value as evidence, it is important to treat all specimens as evidence in terms of care and custody. Stored specimens must be properly marked, tagged, and packaged to ensure the integrity and identity of the items. The individual responsible for specimens and evidence stored in the secured facility should maintain records accurately documenting the evidentiary items and the dispensation of items transferred out of the facility. An example of the record keeping forms is presented in Appendix X4. Forms must be completely filled out and should contain at the minimum the following information: legal case number and title; law enforcement district and official; source of specimen (person, site location (latitude and longitude if available), description of environment where species was found, or other information as appropriate); time and date of collection; descriptions and unique identifiers for samples to be used as evidence; and a complete list of the persons who have had responsibility for guaranteeing the security and integrity of that sample through time (with dated release and receipt signatures for each party). Sealed packages mailed to another individual or the diagnostic laboratory should be marked "EVIDENCE" and "TO BE OPENED ONLY BY (receiving individual's name)". The original chain of custody record should be placed in an envelope marked "CHAIN OF CUSTODY RECORD" and attached to the mailing package. At the end of all diagnostic analyses, the original chain of custody forms should be maintained by the responsible agency with other case-related records.

7.5 *Comprehensive and Systematic Collection of Information and Samples*:

7.5.1 *Initial Off-Site Information*—Upon receiving notification of a fish or wildlife kill, an investigator can often obtain a significant amount of information from the individual reporting the incident on the apparent extent of the incident, whether a field response is necessary, and whether there is potential for spread of the incident. Questions to ask to clarify the nature of an incident and its scale include the following:

7.5.1.1 Where has the event taken place?

7.5.1.2 When did the incident occur and when were dead or disabled (or abnormal) animals observed?

7.5.1.3 What species of animals were affected?

7.5.1.4 How many of each species have died?

7.5.1.5 Did the deaths occur over a short or long period of time?

7.5.1.6 Is mortality on-going?

7.5.1.7 At what rate are the individuals dying?

7.5.1.8 What are the signs of sick or dying animals?

7.5.1.9 Have there been any livestock or other domestic animal deaths?

7.5.1.10 What were the climatic (precipitation, temperature, winds) conditions preceding the incident and have these recently changed?

7.5.1.11 Have there been recent changes in the land use or agricultural practices, or both, (pesticide use, land clearing, large numbers of insects, change in types of crops planted, and so forth) in the area?

7.5.1.12 Has anything similar occurred before in this locality?

7.5.1.13 Any opinion as to what may have caused the incident?

7.5.1.14 Upon answering these questions, the investigator should have enough information to determine whether the incident warrants a field investigation. If the incident does warrant a field investigation, a specific case number should be assigned to the investigation and used on all labels, tags, data sheets, photographs, and other records related to the incident. The investigator must use best professional judgement to determine the intensity of field investigation and the individuals and agencies to contact. When in doubt, consult with experts. Expert opinion regarding fish or wildlife die-offs and effects may be obtained from the NBS Wildlife Health Center, or cooperating laboratories such as the Southeastern Cooperative Wildlife Disease Study, the state wildlife resource management agency, or USFWS law enforcement agents. Names and addresses for state agencies responsible for incident reporting are listed in Appendix X5.

7.5.2 *Initial On-Site Information*:

7.5.2.1 The three rules that govern initiation of any wildlife incident investigation are: protect yourself and others; get the best case history possible; and get the best specimens possible. The handling and collection of specimens in the field will determine what the laboratory can do with them.

7.5.2.2 The investigator's interpretation of the fish and wildlife incident scene will determine the type, number, and location of samples taken and the analyses performed on the samples. Because the first few hours after arrival on the scene of a fish and wildlife incident are the most critical, information should be collected as soon as possible. Some toxicants have very short half-lives; as such, diagnostic signs present at the site (for example, sick or dying animals and water conditions in a flowing stream) may quickly disappear.

7.5.2.3 Immediately upon arrival at the site of the incident, the investigator should quickly survey the scene and record as much information as possible on a standard form (see Appendix X6). This information includes, but is not limited to, date and time of day; location including county, nearby cities or residences, highways, and other significant landmarks; name, address, and phone number of person who first observed or reported the incident and others who can provide on-site information; time when the incident was first observed or reported; estimated time during which the incident occurred; if water is involved, temperature, dissolved oxygen concentration, pH, color, and clarity of the water; weather conditions (temperature, cloud cover, and precipitation) that day and previous night; wind direction and speed; physical condition and types of species involved or seen in the area of the incident; presence of colored (dyed) grains or liquids, open containers or drums, discolored soils, and dead vegetation; if applicable, pesticide, formulation, application method, application rate, specific farm machinery used, and other factors that may influence exposure of animals; recent changes in the number of animals on the area and their source or destination; photographs or videos of the incident site (these should be taken prior to sample collection); and type of land use (agricultural, residential, or riparian) in the area. This information will help narrow the focus of the incident investigation, saving significant time and resources.

7.5.2.4 A detailed description of the physical appearance and behavior of dead, moribund, or sick fish or wildlife is very important. For fish and aquatic invertebrates, record occurrence of flared gills, spinal curvatures, mouths agape, excessive mucus, lesions, frequent bobbing to the water surface, and deliberate avoidance of an area of the water course. For terrestrial species, record occurrence of blood in or around the nose, mouth, or anus; excessive salivation (drooling); sores; evidence of convulsions; abnormal gait or flight behavior; inability to stand or perch; vomiting or diarrhea; and prolapsed penis (in male birds of certain species). It is also important to record the species which are present at the incident site but have not been affected.

7.5.3 *Collection and Handling of Specimens*:

7.5.3.1 It is important that a sample identification system be in place before samples are collected in the field. For samples to play a meaningful role in investigating a fish or wildlife kill, each sample collected should be uniquely identified and related in time and space to the incident, associated to the primary incident number, and accompanied by a chain of custody record. The same unique numbering system for each sample or subsample should be used by all parties dealing with sample collection and processing.

7.5.3.2 In general, plans between analytical facilities and incident response personnel should be in place so that methods for sample preparation and analyses are verified prior to an incident occurring. The investigator should communicate with the analytical agency or group, if possible, before or during sample collection to agree upon the sample types, numbers, and sizes; sample identification system; collection protocols; preservation methods; chain-of-custody requirements; and analyses required.

7.5.3.3 A field log is useful to make entries regarding each sample collected for analysis. Entries should include the sample identification number, the site where collected, the date, and the name or initials of the collector. These entries provide backup identification if sample labels are damaged or lost, or if confusion develops over when and where certain samples were taken.

7.5.3.4 *Labeling*—Individual specimens should be identified so that any other investigator or diagnostician has all the information about the specimens at a glance. The labels should contain unique case number or other identifier; species and date of collection; location where specimens were collected; collector's name, affiliation, and phone number; and name of person who should receive the specimens.

7.5.3.5 *Shipping*—Proper shipping and handling of specimens is necessary to ensure that all specimens arrive at the diagnostic laboratory in good condition. There are restrictions on air shipment of hazardous or flammable substances including dry ice, alcohol, and formaldehyde. Thorough instructions on proper handling and shipping of specimens are provided by Friend **(4)**. Five important rules governing proper shipment of specimens are: prevent cross-contamination of specimens; prevent decomposition of specimens; do not allow specimen fluids to leak; properly identify each specimen; and properly label the package being shipped. Specimens (carcasses or tissues) should be double bagged in plastic and sealed to prevent leakage of fluids and cross contamination with other specimens. Blood, soil, water, or other samples should be placed in leak-proof containers and packaged to prevent breakage if jarred. Specimens must be kept cool during shipping.

7.5.3.6 Interspersing blue ice or other chemical coolants placed outside specimen bags works well.

7.5.3.7 Block ice should be used only if leakage can be avoided. Plastic jugs filled with water and frozen can be used if lids are taped shut to prevent leakage. Use a polystyrene plastic or other insulated cooler for shipping. Coolers at least 1 in. (25.4 mm) thick and with straight sides are sturdier than thinner coolers with tapered sides. Place bagged specimens and coolant inside a plastic bag lining the cooler. The case history and chain of custody can be either placed in a sealed waterproof bag inside the cooler, or placed in an envelope and taped outside the cooler. Use crumpled newspaper to fill any gaps and to keep specimens from shifting during shipping. Coolers then should be placed in a cardboard box or wrapped in cardboard to prevent breakage. Use crumpled newspaper to keep the cooler from shifting in the cardboard box. Seal the package with strapping tape and ship overnight delivery to the diagnostic laboratory. Be sure to notify the receiving laboratory before shipping to ensure that someone will be available to receive the package and process it in a timely manner. If the package contains biological specimens, the words" Fish/ Wildlife Diagnostic Specimens" should be marked on the outside of the container.

7.5.4 *Water and Sediment*:

7.5.4.1 A complete set of water chemistry analyses should be conducted to help rule out other possible causes and help identify any contributing factors (for example, dissolved oxygen, pH) that could influence the toxicity of the suspected chemical agents. Analyses that should always be run as soon as possible are (in approximate order of importance) dissolved oxygen, pH, temperature, ammonia, alkalinity, conductivity, nitrite and nitrate, total suspended solids, sulfate, turbidity, and salinity (if marine or estuarine). Dissolved oxygen and temperature measurements must always be done in-situ; the other parameters can be done in the laboratory. Other less important analyses include: biological oxygen demand, total organic carbon, chemical oxygen demand, and hardness. There are accepted standard methods **(18,23,24)** for most of these water analyses.

7.5.4.2 If the probable cause of a die-off is believed to be a toxic substance or substances, then the next step is to establish whether the suspect chemical was present in sufficient quantity to be toxic.

7.5.4.3 *Water*—For potential water pollution from a suspected source, collect at least three samples (upstream, source, and downstream) to ascertain if the discharge from the suspected source has caused the observed fish or wildlife incident. It is beneficial to perform chemical analyses on the water samples. Toxicity analyses can be beneficial in substantiating the degree of toxicity at the site for comparison with known toxicity levels of chemicals.

7.5.4.4 Sampling protocols should be in place before investigative sampling is begun. The specific types of sampling and analyses needed must be determined on a case-by-case basis by the on-site investigator. As many samples as convenient should be taken over the area. Although it may not be necessary to have all samples analyzed, there may not be another opportunity to collect useful samples. Before sample bottles are filled, each bottle should be rinsed two or three times with the water that is being sampled (unless the bottle contains a preservative or dechlorinating agent). Preferably, water samples should be refrigerated at a temperature of 4°C in amber bottles and stored in darkness.

7.5.4.5 The minimum volume needed for water samples varies with the type of analysis to be performed on the sample. In general, a 1-L sample per site is sufficient. Toxicity tests may require much larger sample volumes. It is important that properly cleaned and prepared containers be used to collect and store the samples. In general, samples to be analyzed for inorganic compounds can be taken with plastic (polyethylene or equivalent) bottles that have been acid washed and rinsed with distilled water. For preservation, samples taken for analyses of metals should be acidified to pH 2 with redistilled nitric acid. Samples taken for suspected pesticides or other toxic organic will require amber glass bottles with TFE-fluorocarbon-lined caps. The glass bottles should have been rinsed with hexane and dried before use. If volatile organic are suspected, the sample bottles with Septa-liners should be filled underwater and capped, leaving no air space. Properly cleaned sample bottles and containers are commercially available. Ampules that contain premeasured amounts of acid for preservation of water samples are also commercially available. Use of these ampules reduces acid leaks in sampling kits.

7.5.4.6 For streams more than about 60 m wide, samples should be taken at two or more points along a transect across the stream. In large water bodies, it may be necessary to take samples at various depths and locations to get representative conditions at the incident site. Sampling devices that can be used to take water samples are outlined by EPA **(23)**, Hill **(20)**, and APHA, et al **(17)**.

7.5.4.7 *Sediment*—It may not be necessary to collect sediment samples in all fish-kill investigations. However, samples should be consistently taken from the same sites where water samples were taken (above, within, and below the incident area). Special sampling sites below point source inputs may be desirable and should always be carefully documented. The method of handling the samples after collection and before analysis is determined by the type of test. Samples should always be kept cool (4°C) or frozen and stored at a temperature of −20°C or lower **(25,26)**.[9] However, if samples are to be used in toxicity tests, they should always be kept cool (4°C), but never frozen.

7.5.4.8 Sediments are usually taken with a corer or mechanical grab dredge **(23,25,26)**. The needed sample size is

[9] For example, see Guides E 1391 and E 1525.

usually not less than 50 g. One-quart (0.95-L) widemouthed glass jars with screw-cap lids are acceptable containers. The caps should be lined with TFE-fluorocarbon sheeting (metal analysis) or aluminum foil (organic analysis). All jars, lids, sheeting, or foil should first be washed with a nonphosphate, laboratory-grade detergent, and triple rinsed with tap water. They should then be rinsed with reagent grade nitric acid (1:1) and tap water, followed by a rinse with 1:1 hydrochloric acid (reagent grade), and a triple rinse with distilled water. The containers and materials should then be rinsed with acetone, followed by pesticide-grade hexane, and dried in a contaminant-free area. Commercially prepared containers are available. The core depth should be documented because some pesticides accumulate within the top 2.5 cm of the sediment.

7.5.5 *Fish and Wildlife*:

7.5.5.1 Tissue samples for histological examination should not be frozen. Freezing disrupts cells and makes the specimens worthless for histological examination. It is imperative that tissue specimens be placed into a suitable fixative as soon as possible, preferably at a ratio of one part tissue to ten parts fixative. A 10 % solution of buffered neutral formalin is readily available and is an acceptable fixative. Check with the histopathologist who will do the tissue analyses for his or her choice of fixative and for other instructions on fixation techniques. If euthanasia has been carried out using a chemical, it is vital that the information as to the compound used is relayed to the analytical laboratory to minimize confusion on analysis.

7.5.5.2 *Wildlife*—Rotten carcasses are usually of no diagnostic value in necropsies but may have value for residue analyses. For example, certain poisonous substances (for example, lead) can be detected in rotten carcasses. A rotten carcass of a threatened or endangered species may be worth submitting for documentation. Scavenged or otherwise damaged carcasses may also be of little diagnostic value. When in doubt, consult with the diagnostician as to whether the carcass will be useful. The preferred specimen is a whole carcass. An ideal specimen is one that you've observed sick and then euthanized or let die. Whole carcasses are preferred so that the diagnostician is able to examine the entire animal in order to identify and evaluate all the physiological changes that may have occurred preceding the animal's death. If laboratory tests are required, such as those for isolation of bacteria or viruses, the diagnostician will know which organs or tissues to submit and will be able to remove them with a minimal amount of contamination. Tissues removed during a field necropsy by untrained personnel can become too contaminated to do any meaningful bacteriological or virological work.

7.5.5.3 Recently dead specimens should be placed in plastic bags and refrigerated at a temperature of 4°C. Specimens should be cooled on site if possible by placing the bagged specimen in ice or blue ice in an insulated container. If specimens cannot be submitted to a laboratory within 24 to 48 h, they should be placed in a freezer at the lowest possible temperature. When freezing specimens, be sure that they are cooled quickly. Do not put specimens that are warm in an insulated, closed container and then place the closed container in the freezer. If an especially large number of carcasses is involved, you may need to dispose of carcasses on site. Friend **(4)** provides excellent guidance on disposal of carcasses for controlling a disease outbreak. In pesticide poisoning cases, efforts should be made to remove or otherwise make poisoned carcasses unavailable to scavengers, that may become poisoned by eating contaminated carcasses. The species, age, number, and sex of any carcasses collected and disposed of or animals observed sickened should be documented. This information is valuable in determining the minimum impact of the incident and may be useful should the case be prosecuted.

7.5.5.4 *Fish and Aquatic Invertebrates*—A representative size series of moribund or recently dead fish of each species affected should be collected. If possible, healthy fish of the same species and size from an unaffected area should also be collected to provide reference data. Methods that are used to preserve the various samples should always be noted on the label. For samples to be analyzed for pesticides or other toxic organic substances, the whole fish should be rinsed with clean water, wrapped in aluminum foil (with the dull side toward the specimen), and frozen as quickly as possible. Samples to be analyzed for metals or other elements should be collected separately, placed in polyethylene bags, and frozen. Subsamples of tissues such as brain, gills, or blood that are needed for special analyses should be taken immediately after sampling and frozen in separate clean glass containers.

7.5.5.5 Tissues for histological examination should be taken from moribund fish, never from dead fish (in which postmortem changes are likely to have occurred). Fish that have been dead longer than 10 to 15 min are not suitable specimens. Fish tissues that were preserved in a fixative for histological examination should be transferred to 70 % ethanol for storage. They can then be held for a year or more if the solution is renewed periodically. For further information, see Morrison and Smith **(27)** or Yasutake **(28)**.

7.5.5.6 For analytical purposes, it is better to collect several small fish than one large fish from each species that is affected. The numbers collected, amount of tissue needed, and preservation techniques depend on the types of analyses to be performed. If no animals are found alive, select the freshest specimens. The sample should reflect the size range and species composition of the affected population. If fish cannot be preserved in the field, they should be bagged or wrapped in plastic and placed in wet ice.

7.5.5.7 Samples of benthic invertebrates can be used to determine the extent of the incident and to document recovery after the incident. Samples should be taken in the same areas in which water and sediment samples were taken. If sufficient invertebrates, especially bivalves, are available, tissue can be used for residue analyses. Tissue samples should be frozen in a suitable clean container and properly tagged and labeled.

7.5.5.8 In most investigations, benthic invertebrate samples are not needed for toxicant residue analyses. If information on residues in the benthos is desired, a sample of at least 100 g is required for analyses. Generally, large invertebrates such as crayfish or bivalves suffice as samples for analytical purposes. Samples should be frozen in the same type of prepared containers as those used for sediments and stored at a temperature of −20°C until they are analyzed.

7.5.5.9 Zooplankton samples can also be used to document the nature of the cause and the extent of the incident. The presence or absence of live animals can be useful information in determining the cause of the incident. The choice of sampling gear used to collect the zooplankton depends on the types of organisms present and the body of water to be investigated. To collect zooplankton, 30 L of water are filtered through an 80-µm mesh plankton net. For a discussion of sampling techniques, see APHA, et al **(17)** or Weber **(28)**. To preserve zooplankton, use 70 % isopropyl alcohol or 5 % buffered formalin. Do not store the sample in formalin longer than 48 h before transferring it to 70 % isopropyl alcohol.

7.5.6 *Vegetation and Soil*—It is helpful with suspected pesticide incidents to analyze the adjacent crops and soils for pesticides. Plant material should be collected and stored using methods similar to those used for fish and wildlife (see 7.5.3.2). The entire plant, including the rootball is desirable for analysis; if this is impractical, remove parts of the foliage with the greatest surface area (for example, leaves). Similarly, soil should be collected and stored using methods used for sediment (see 7.5.3.1). A small stainless steel trowel can be used to collect the top 2 to 4 in. (50.8 to 101.6 mm) of soil. The trowel should be cleaned and rinsed in hexane between samples.

7.6 *Analyses of Samples*:

7.6.1 *Fish*—For fish, the best tissue for analysis to establish exposure is the gill. For inorganic analyses, at least three fish, or as many as needed to provide 100 g of whole body tissue as the minimum total sample, is required. Preferably, collect three samples for each species from each site. For organic analyses, at least three fish, or as many as needed to provide 250 g of whole body tissue as the minimum total sample, is required. Check with your laboratory and establish the minimum quantity of tissue they need for analysis. Analyses of water, vegetation, soil, and sediment from the incident site will help to establish exposure.

7.6.2 If it is suspected that the causative agent is a volatile substance, about 100 g of tissue should be placed in containers that can be sealed airtight and frozen. The composite samples of three or more fish should be separately wrapped in foil and placed in a single bag, properly labeled, and frozen. Samples of all types should be frozen as quickly as possible and kept frozen at a temperature of −20°C or lower until analyzed. For a large kill with many species, the investigator must select the species to be collected. Samples should include representatives from all trophic levels that are affected (that is, herbivores, omnivores, forage fish, and predators). It is critical that the same species of fish (and preferably of the same sizes) be sampled from the control or reference area as from the incident area. The number of samples collected will depend on the extent of the kill, the number of species involved, agency protocol, instructions from the analytical facility, and the estimated costs of analyses.

7.6.3 *Wildlife*—For birds, the best tissues to establish oral exposure to an acute toxicant are the contents of the crop/proventriculus and the gizzard or stomach. For living birds, a foot wash with methanol or isopropyl alcohol and analysis of contour (body) feathers are very useful for establishing exposure to an aerosolized chemical application. If the bird is dead, removal and analysis of the skin from the sole of the bird's feet can be substituted for the use of a footwash. Typically, a footwash must be performed within 48 to 72 h of exposure to detect the presence of the chemical. After 48 to 72 h, most of the material has been absorbed into the skin. Liver tissue, fat deposits, and brain tissue are generally considered best for identifying the presence of toxic levels of trace elements (lead, mercury) and many of the fat soluble chemicals like PCBs and the organochlorine pesticides. Feather and hair samples are often used as non-invasive samples to detect chronic exposure to heavy metals which may be contributing to an overall decline in fitness of the animals thus making them more susceptible to disease or other environmental conditions. As with fish, analysis of water, sediment, vegetation, and soil samples from the incident area will also help to establish routes of exposure and identify occurrences of exposure to multiple toxic materials. In the case of predators and scavengers, it may be necessary to collect local prey species or scavenged carcasses to determine exposure.

7.7 *Interpretation of the Data*:

7.7.1 Incident reports are instrumental in the regulatory arena for identifying or confirming risk to fish and wildlife. Incident reports can identify particularly sensitive species or pinpoint geographic areas of potentially high risk. Incident data can identify the potential impact of a pesticide on fish and wildlife for pesticide registration activities. The following types of information should be incorporated into the incident review:

7.7.1.1 The certainty that the pesticide caused the incident (based on established criteria (for example, residue analysis, cholinesterase measurements, and so forth));

7.7.1.2 The use pattern and how similar the use pattern was to other use patterns which have caused problems;

7.7.1.3 Magnitude of effect (for example, total numbers of birds impacted, one or thousands, as well as the potential to effect the population of a species, such as one raptor versus one starling);

7.7.1.4 Protection of affected species by federal or state regulation (for example, Migratory Bird Treaty Act, Endangered Species Act);

7.7.1.5 Location of incident (for example, did the incident occur at a site similar to other documented incidents such as corn in Illinois versus corn in Ohio);

7.7.1.6 Timing of application (for example, did the incident occur immediately following application, or did it occur several months later);

7.7.1.7 Number of incidents for the pesticide being evaluated (for example, is there one incident or several, indicating a trend).

7.7.1.8 Factors that are being considered to be incorporated into the risk assessment include: the reporting rate for the individual states and usage data (background rate of incidents on a national basis and by state). The ultimate outcome is to estimate the probability that an incident would be repeated under those same set of circumstances.

7.7.2 *Fish*—Canada **(29)** and American Fisheries Society **(11)**, and Meyer and Ellersieck **(12)** have published summaries of acute and chronic fish toxicity information and have developed criteria recommendations for many pollutants. In addition, U.S. EPA develops water quality standards that are available for each state as a separate document or as part of a compilation in one document that can be purchased from the National Technical Information Service. Exceeding criteria, objectives, or standards may not necessarily result in an incident.

7.7.2.1 Laboratory and field studies have shown that many factors influence the toxicity of chemicals to fish. The origin of modifying factors may be either biotic or abiotic **(12,30)**. Biotic factors include species, life stage and size, nutritional state, general health, and parasitism. Abiotic factors include characteristics of the water (for example, temperature, pH, hardness, alkalinity, osmolality, dissolved oxygen, salinity, dissolved organic carbon), possible binding to suspended or dissolved materials, and formulation of pesticide products.

7.7.2.2 Water hardness has little effect on the toxicity of organic compounds. However, increased water hardness (as calcium and magnesium) can reduce the availability of metals such as aluminum, cadmium, mercury, and copper **(30-32)**. Alkalinity and pH (in addition to hardness) also influence the availability of metals, such as copper **(30)**. Hydrogen ion concentration (measured as pH) influences the toxicity of chemicals that ionize. For example, the toxicity of ammonia, cyanide, and hydrogen sulfide is influenced by the pH of the water. Unionized molecules usually are more lipid soluble than ionized forms and thus penetrate membranes more readily **(33,34)**. As noted by Mayer and Ellersieck **(12)** in a study of 410 chemicals, pH affected the toxicity of only about 20 % of the organic chemicals tested, but caused greater changes in 96-h LC50 values than any of the other water chemistry factors examined.

7.7.2.3 It is important to establish some measure of the relative toxicity at the site. A valid pH measurement may be sufficient to establish whether the hydrogen ion concentration was lethal. In extremely soft water, pH determinations should be made with a special electrode designed for use in waters of low ionic strength. Most substances are toxic to organisms if the concentration is high enough and the length of exposure is long enough. Although data obtained from 24-h exposures are most appropriate for use in evaluating an acute kill situation, data from 24, 48, and 96-h tests can also be used to estimate the toxicity of a substance suspected of causing the kill. The 95 % confidence interval establishes a range for the LC50 and is helpful in determining whether the concentration of the chemical found in the field was high enough to cause acute toxicity **(12)**.

7.7.3 *Wildlife*—Acute toxicity levels of pesticides for birds and mammals (oral LD50 values) are categorized as relatively nontoxic (>2000 mg/kg body weight (bw)), slightly toxic (500 to 2000 mg/kg bw), moderately toxic (50 to 500 mg/kg bw), highly toxic (10 to 50 mg/kg bw), and extremely toxic (<10 mg/kg bw).

7.7.3.1 As with fish, there are many factors that can influence the toxicity of a chemical or group of chemicals. Biotic factors can include species, feeding behaviors of the species exposed, age (life stage), and physical condition of the animals impacted.

7.7.3.2 Abiotic factors influencing toxicity can include the mechanism of exposure (ingestion, dermal contact), primary or secondary exposure, method of application of the chemical, physical state of the chemical (granular versus liquid), other materials used in combination (as synergists or sticking agents), time of year and weather conditions, and availability of alternate food or water supplies. The extent to which a chemical can be absorbed or stored by various tissues in an animal's body, or both, can greatly influence the extent to which adverse effects of exposure will be observed immediately following exposure versus delayed effects. Fat soluble materials may be stored in mesenteric or subcutaneous fat deposits until the animal is stressed significantly at which time the chemical may be mobilized and result in death or observable impairment.

7.7.4 When investigating a wildlife incident, it is important to determine as many of the potential abiotic factors that could influence toxicity as possible. These may not seem significant at the time but they can play a critical role in interpreting sample analysis results and may provide clues to chemical exposures that may not be readily apparent.

7.8 *Determining the Significance of Investigation*:

7.8.1 If toxic concentrations of a chemical are present at the incident site, exposure of the wildlife to the chemical has been confirmed, and conditions at the incident site are consistent with other incidents associated with that chemical, then it is likely that the chemical caused the incident. Sometimes, not all three conditions occur and it is less likely that the chemical was responsible.

7.8.2 The significance of the detected residues must be determined. The presence of residues may indicate non-lethal exposure. Factors that must be considered include any physical or biological breakdown of the toxic material or its elimination from the body, or both. These factors include the following:

7.8.2.1 How long the animal survived from exposure until death.

7.8.2.2 How long from death to analysis.

7.8.2.3 The physical conditions in which the animal was found and subsequently kept prior to analysis (for example, temperature).

7.8.2.4 The compound detected and its physical properties, breakdown products, and behavior in animal systems.

7.8.2.5 The state of the animal (stressed from breeding, migrating, starvation, molting, bad weather conditions, and so forth).

7.8.2.6 The likelihood that the species found are carrying background residues.

7.8.2.7 The confidence in the detection method for finding all or some of the residues.

7.8.2.8 The relevance of the residue of the compound in the tissue in which it was detected.

7.8.2.9 The presence or absence of residues of breakdown products in the tissues.

7.8.2.10 The presence or absence of supportive postmortem findings (for example, small residues of anticoagulants with postmortem findings of large amounts of hemorrhaging may be more significant than a larger residue with no hemorrhaging found).

8. Reporting of Incidents

8.1 *Format of Report*:

8.1.1 There are basic sets of information that are useful to state and federal agencies and certain industries. The information most useful about an investigation include the case number (assigned by the reporting party); agency or contact reporting the incident; state and county of incident (latitudes and longitudes would be helpful); date the incident started and ended; total number of nontarget species reported to have been affected; magnitude of effect (for example, size of incident area); weather conditions (rainfall, wind conditions, and so forth) at time of incident; cause of incident; each species affected; habitat in which the species were found; response of the organism(s), including mortality and behavioral changes; age of the adversely affected organism(s); total number affected of each species; number necropsied; necropsy condition of each organism; the cholinesterase range of the species that have been analyzed, as well as the number analyzed; results of the tissue analysis; environmental analysis including the dissolved oxygen level, pH of the water, and residues of the contaminant in water, soil, sediment, and foliage; a general discussion of the conditions under which the incident occurred; and reference to the laboratories conducting the analysis.

8.1.2 In addition to the preceding, if the incident has been determined to have been caused by a pesticide, then additional data are needed. These include specific pesticide(s) implicated, formulation of pesticide, the crop or site on which the pesticide was applied, whether the pesticide application was a registered or a misuse (for example, baiting carcasses), the method of application used for the pesticide, the application rate at which the pesticide was applied, and the distance from the treatment site to where the affected organism(s) was found (for example, bird was found 100 ft (30.5 m) from a cotton field treated with the pesticide).

8.1.3 The preceding information may be used by a variety of governmental personnel, including law enforcement investigators, risk assessors, and the risk or resource managers. These data assist the risk assessors in weighing the evidence in an ecological risk assessment.

8.2 *Documentation of Information*:

8.2.1 It is important that records of incident investigations be as complete and factual as possible. The degree of certainty about the cause of an incident should be made. It is important that the reporting of the incident reflect the degree of uncertainty about the cause of death. This information is important in both the development of a legal case and in determining the weight of the evidence in an ecological risk assessment **(35)**.

8.2.2 To standardize the documentation requirements for fish and wildlife incident investigations, the development of standard operating procedures (SOP) should be used. The SOPs are valuable to specifically define the procedures that should be followed in all investigations, the information necessary for documentation of incident parameters, and the requirements for collecting, analyzing, and storing of data and samples related to the investigation. The completeness of documentation will be a large determinant in the usefulness of incident information in management and regulatory decisions.

APPENDIXES

(Nonmandatory Information)

X1. EQUIPMENT TO PROTECT HUMAN BODY AGAINST CHEMICAL HAZARDS

X1.1 Equipment to protect the body against contact with known or anticipated chemical hazards has been divided into four categories according to the degree of protection afforded:

X1.1.1 *Level A*—Should be worn when the highest level of respiratory, skin, and eye protection is needed;

X1.1.2 *Level B*—Should be selected when the highest level of respiratory protection is needed, but a lesser level of skin protection. Level B protection is the minimum level recommended on initial site entries until the hazards have been further defined by on-site studies and appropriate personnel protection utilized;

X1.1.3 *Level C*—Should be selected when the type(s) of airborne substance(s) is (are) known, the concentration(s) is measured, and the criteria for using air-purifying respirators are met; and

X1.1.4 *Level D*—Should not be worn on any site with respiratory or skin hazards. It is primarily a work uniform providing minimal protection.

X1.2 *Level A Protection—Personnel Protective Equipment:*

X1.2.1 Pressure-demand, self-contained breathing apparatus, approved by the Mine Safety and Health Administration (MSHA) and National Institute of Occupational Safety and Health (NIOSH),

X1.2.2 Fully encapsulating chemical-resistant suit,

X1.2.3 Coveralls,[10]

X1.2.4 Long cotton underwear,[10]

X1.2.5 Gloves (outer), chemical-resistant,

X1.2.6 Gloves (inner), chemical-resistant,

X1.2.7 Boots, chemical-resistant, steel toe and shank. (Depending on suit construction, worn over or under suit boot),

X1.2.8 Hard hat[10] (under suit),

X1.2.9 Disposable protective suit, gloves, and boots[10] (worn over fully encapsulating suit), and

X1.2.10 Two-way radio communications (intrinsically safe).

X1.2.11 The fully encapsulating suit provides the highest degree of protection to skin, eyes, and respiratory system if the suit material is resistant to the chemical(s) of concern during

[10] Equipment is optional.

the time the suit is worn or at the measured or anticipated concentrations, or both. While Level A provides maximum protection, the suit material may be rapidly permeated and penetrated by certain chemicals from extremely high air concentrations, splashes, or immersion of boots or gloves in concentrated liquids or sludges. These limitations should be recognized when specifying the type of chemical-resistant garment. Whenever possible, the suit material should be matched with the substance it is used to protect against.

X1.2.12 Many toxic substances are difficult to detect or measure in the field. When such substances (especially those readily absorbed by or destructive to the skin) are known or suspected to be present and personnel contact is unavoidable, Level A protection should be worn until more accurate information can be obtained.

X1.3 *Level B Protection—Personnel Protective Equipment:*

X1.3.1 Pressure-demand, self-contained breathing apparatus (MSHA/NIOSH approved),

X1.3.2 Chemical-resistant clothing (overalls and long-sleeved jacket; coveralls; hooded, one- or two-piece chemical-splash suit; disposable chemical-resistant coveralls),

X1.3.3 Coveralls,[10]

X1.3.4 Gloves (outer), chemical-resistant,

X1.3.5 Gloves (inner), chemical-resistant,

X1.3.6 Boots, chemical-resistant, steel toe and shank,

X1.3.7 Boots (outer), chemical-resistant (disposable, worn over permanent boots),[10]

X1.3.8 Hard hat[10] (face shield), and

X1.3.9 Two-way radio communications (intrinsically safe).

X1.3.10 Level B equipment provides a high level of protection to the respiratory tract, but a somewhat lower level of protection to skin. The chemical-resistant clothing required in Level B is available in a wide variety of styles, materials, construction detail, permeability, and so forth. These factors all affect the degree of protection afforded. Therefore, a specialist should select the most effective chemical-resistant clothing (and fully encapsulating suit) based on the known or anticipated hazards or job function, or both.

X1.3.11 For initial site entry and reconnaissance at an open site, approaching whenever possible from the upwind direction, Level B protection (with good quality, hooded, chemical-resistant clothing) should protect response personnel, providing the conditions described in selecting Level A are known or judged to be absent.

X1.4 *Level C Protection—Personnel Protective Equipment:*

X1.4.1 Full-face, air purifying, canister-equipped respirator (MSHA/NIOSH approved),

X1.4.2 Chemical-resistant clothing (coveralls; hooded, two-piece chemical-splash suit; chemical-resistant hood and apron; disposable chemical-resistant coveralls),

X1.4.3 Coveralls,[10]

X1.4.4 Gloves (outer), chemical-resistant,

X1.4.5 Gloves (inner), chemical-resistant,[10]

X1.4.6 Boots, chemical-resistant, steel toe and shank,

X1.4.7 Boots (outer), chemical-resistant (disposable, worn over permanent boots),[10]

X1.4.8 Hard hat (face shield),[10]

X1.4.9 Escape mask,[10] and

X1.4.10 Two-way radio communications (intrinsically safe).

X1.4.11 Level C protection is distinguished from Level B by the equipment used to protect the respiratory system, assuming the same type of chemical-resistant clothing is used. The main selection criterion for Level C is that conditions permit wearing air-purifying devices.

X1.4.12 Total unidentified vapor/gas concentrations of 5 ppm above background require Level B protection. Only a qualified individual should select Level C (air-purifying respirators) protection for continual use in an unidentified vapor/gas concentration of background to 5 ppm above background.

X1.5 *Level D Protection—Personnel Protective Equipment:*

X1.5.1 Coveralls,

X1.5.2 Gloves,[10]

X1.5.3 Boots/shoes, leather or chemical-resistant, steel toe and shank,

X1.5.4 Boots, chemical-resistant (disposable worn over permanent boots),[10]

X1.5.5 Safety glasses or chemical splash goggles,[10]

X1.5.6 Escape mask,[10]

X1.5.7 Level D protection is primarily a work uniform. It should be worn in areas where: only boots can be contaminated, or there are no inhalable toxic substances.

X1.5.8 Personnel should not eat, drink, or smoke during or after sampling until after decontamination steps are taken.

X1.5.9 Sampling personnel should be trained in safety aspects of hazardous waste sampling.

X2. COMMON CAUSES OF FISH AND WILDLIFE INCIDENTS

X2.1 *Fish Incidents:*

X2.1.1 *Chemical Agents*:

X2.1.1.1 *Acids*—Acidity is a measure of the hydrogen ion concentration of water and is an indicator of a combination of substances and conditions of the water. It is usually caused by the presence of free carbon dioxide, mineral acids such as sulfuric, weakly dissociated acids such as phosphoric, and salts of strong acids. Acid conditions in surface waters may be attributable to natural causes including humic acids, or to industrial wastes such as pickling liquors, effluent from the manufacture of explosives, acid-mine drainage, or sulfate waste liquors. Freshwater and marine aquatic life live within the pH range from 6.5 to 9.0 **(11)**. At the pH of 5.0 to 6.0, the water is unlikely to be harmful to any species unless the free CO_2 is greater than 20 ppm or the water contains iron salts. However, the toxicity of several common pollutants including metals, HCN, ammonia, and H_2S are markedly affected by pH. An acid discharge may liberate sufficient carbon dioxide from bicarbonate in the water to be directly toxic. Generally, aquatic life can not tolerate pH values much less than 4.0. The EPA has developed criteria for aquatic life for pH (PB89-141527).

X2.1.1.2 *Trace-Metals*—Copper, zinc, mercury, cadmium, iron, chromium, lead, and nickel are common metal contaminants from mining, radiator shops, and electroplating industries. Metals are generally very toxic to aquatic life. Concentrations (µg/L) in excess of 3.9 Cd, 18 Cu, 1000 Fe, 83 Pb, 16 Cr, 170 Zn, 2.4 Hg, and 1400 Ni can be lethal to fish and sensitive aquatic invertebrates. Generally, the toxicity of metals is dependent on water pH, hardness, and alkalinity, with metals being more toxic in softer, less alkaline or lower pH waters. The EPA has developed criteria for aquatic life for cadmium (PB89-141469), chromium (PB89-141584), copper (PB89-141592), iron (PB89-141543), lead (PB89-141626), mercury (PB89-141378), and zinc (PB89-141519).

X2.1.1.3 *Nitrogenous Materials*—Ammonia, nitrate, and nitrite are found in industrial wastes, municipal wastewater effluents, and agricultural discharges, including feedlot runoff. The unionized form of ammonia is toxic and the percentage of un-ionized ammonia is controlled by water temperature and pH. Lethal concentrations of un-ionized ammonia to fish varies from 0.2 to 2.0 mg/L. The EPA has developed criteria for aquatic life for ammonia (PB81-227144), nitrite, and nitrate (PB26-3943; PB89-141618).

X2.1.1.4 *Chlorine*—Chlorine is a major constituent of sewage and industrial processes that use chlorine for bleaching operations or to control organisms that grow in cooling water systems. The disinfectant, which denatures animal and plant tissue, is extremely toxic to aquatic life. The toxicity to aquatic life of chlorine will depend upon the concentration of total residual chlorine, which is the amount of free chlorine (chlorine gas and hypochlorite ion) plus chloramines (mono and dichloramines). Chlorine residuals above 10 µg/L can be lethal to sensitive fish (salmonids) and concentrations above 70 µg/L will kill even less sensitive species. The EPA has developed criteria for aquatic life for chlorine (PB85-227429).

X2.1.1.5 *Pesticides*—The OPP at EPA has documented that state agencies annually receive about 3800 reports of pesticide-related fish, wildlife, and plant incidents. The use of pesticides poses risk to nontarget fish, wildlife, and plant species. Approximately 2500 incidents have been reported to EPA. Based on the data collected by EPA, insecticides cause the greatest number of fish- and bird-kill incidents, when compared to herbicides and fungicides. Of the insecticides, organophosphates appear to cause the greatest number of fish kills when compared to other classes of insecticides (for example, carbamates).

X2.1.1.6 Aside from chlorine, that is technically a pesticide, most fish kills in California over the last ten years due to pesticides have been caused by the insecticide endosulfan, canal herbicide acrolein, and general aquatic herbicide use of copper sulfate.

X2.1.1.7 Polycyclic aromatic hydrocarbons (PAHs) constitute a broad class of contaminants that are widely distributed in the aquatic environment. The PAHs are produced by the combustion of organic materials and are components of coal and oil. Oil spills are the primary incident associated with PAHs, however releases of oil may occur periodically with discharges of effluents and storm sewers. Oil generally has low water solubility. Therefore, during a release, a portion of the oil forms a layer on the surface of the water and a portion sinks into the water column and adheres to surfaces including sediments, plants, and organisms. Fish and invertebrate mortality occurs due to interference in respiration as oil and oil emulsions cover respiratory structures.

X2.1.2 *Biological Agents*:

X2.1.2.1 Outbreaks of disease are seldom the result of a single factor. Three factors are involved in every potential disease situation: susceptible hosts, pathogenic organisms, and predisposing environmental conditions. All must be present when an epizootic event occurs. Snieszko **(15)** listed decreased immunological response, poor genetic resistance, temperature stresses, pollution, unfavorable water chemistry, and other adverse conditions as some of the possible predisposing factors. Adverse conditions may include factors such as crowding, inadequate food supply, spawning activity, storms, and seasonal changes.

X2.1.2.2 *Viral Agents*—Viruses have seldom been documented as causes of major fish kills in nature but can infect very early life stages of fish. Major kills of fry and fingerlings may occur without visible evidence.

X2.1.2.3 A fish kill investigator who is not associated with a laboratory routinely working with fish cell cultures will be unable to process samples for virological assay at the kill site. Instead, the investigator should select and properly package fish for shipment to a laboratory equipped to isolate and identify fish viruses. Moribund animals showing lesions and aberrant behavior should be selected for analysis.

X2.1.2.4 *Bacterial Agents*—Most bacterial diseases of fish are stress-related. Usually the stressful, but sublethal, situation occurred 10 to 14 days before the start of the epizootic. An investigator should be alert for seasonal stresses related to climate or weather or to normal physiological changes in fish, such as those related to migration or spawning.

X2.1.2.5 Massive winter and spring incidents involving gizzard shad are classic examples of fish kills associated with the bacterium *Aeromonas hydrophila*. This organism is a ubiquitous facultative pathogen that frequently causes disease when the defense systems of fish are compromised by stressful environmental conditions, nutritional deficiencies, low temperatures, or reduced winter feeding. Symptoms of this disease include hemorrhages and hyperemic (red) areas on the body, fins, and internal organs of affected fish.

X2.1.2.6 *Flexibacter columnaris* causes disease in cultured and wild fishes. It is a serious problem in migrating salmon of the Pacific Northwest, particularly where dams have transformed the rivers into a series of lakes, warmed the water, and otherwise modified the environment to favor this bacterium. It may also cause mortality among other species during the spring spawning season. Fish infected by this pathogen have grayish lesions on the fins or body that progressively destroy the skin and gills.

X2.1.2.7 *Fungal Agents*—Fungal agents rarely cause major fish kills in nature. If fish are injured, diseased, or die of any cause, fungi rapidly invade the lesions or carcass and may lead an investigator to ascribe greater significance to the fungal growths than they warrant. Fungi are also opportunistic, secondary invaders around lesions caused by injuries, bacteria, or parasites.

X2.1.2.8 *Parasitic Agents*—Parasites are generally not the cause of major fish kills in natural waters. Their primary effect is to act as stressors: parasites may render fish vulnerable to secondary infections or weaken their tolerance of environmental changes. *Ichthyophthirius multifiliis* (Ich) is a ubiquitous, freshwater parasite that shows no host specificity. The parasites are seen as white spots under the epithelium of the fins, body, and gills. In the wild, kills of fish caused by Ich usually occur in ponds or lakes, but epizootics have been reported in rivers. Ich infestations are most common in late winter and early spring when fish are still in relatively poor condition due to the stresses of overwintering.

X2.1.2.9 It is often difficult to identify viral, bacterial, fungal, or parasitic agents in the field. Specific guidance for sample preservation should be obtained from the analytical facility. In general, the fish should be bagged or wrapped in plastic, packed with wet ice, and transported to the laboratory as soon as possible. The fish must not be frozen.

X2.1.2.10 *Toxic Algal Blooms*—Some blue-green algae and certain dinoflagellates release toxins that kill or inhibit other algae. When competition for nutrients becomes intense, the level of toxin released climbs. Eventually, the water may become toxic to zooplankton, insects, fish, and sometimes even to animals that drink the water. Mortalities due to toxic algal blooms are unique in that production of the toxin is strongly related to photosynthetic activity. Incidents occur between 9:00 a.m. and 4:00 p.m. Often there is a large-scale die-off of the problem alga, sometimes followed by signs of a classical oxygen depletion. Red tides, that occur in marine waters because of blooms of the dinoflagellate *Gymnodinium brevis*, are a common example. In addition to bluegreens (Cyanophyta) and certain dinoflagellates, other algae, including diatioms have been now shown to produce toxins, for example, domoic acid by *Pseudonitzschia*, free radicals by *Chattenella*, *Heterosigma* by unknown means, and the diatom Chaetoceros (by physical gill damage). A lot of these are marine, including the extensive marine fish kills by algae in the United States and by Chrysochromulina in Norway **(36,37,38)**.

X2.1.3 *Physical Agents*:

X2.1.3.1 *Oxygen Depletion*—Perhaps the most common natural cause of fish kills is oxygen depletion. It occurs when the total demand for oxygen by biological and chemical processes exceeds the oxygen input from aeration and photosynthesis or when the water is unable to hold sufficient dissolved oxygen to maintain aquatic life through the night. Oxygen depletion is usually associated with abundant growth of rooted vegetation, heavy algal blooms, or high concentrations of organic matter. Oxygen depletion is usually seasonal in occurrence unless there is a release of organic nutrients such as that resulting from untreated or partly treated sewage. Oxygen depletion in natural waters is most common during June, July, and August. The environmental evidence associated with summer oxygen depletion may include kill occurred abruptly in early morning, usually before sunrise; large fish died first and small fish may be alive; species with the highest oxygen requirements die first; dissolved oxygen concentration is low, usually less than 1 ppm; the pH is between 6.0 and 7.0; concentration of free carbon dioxide is high; color of the water changes from light green to pea-soup green, brown, gray, or black; and decaying vegetation and algae (black and odorous) may be abundant.

X2.1.3.2 *Turnovers*—Occasionally, weather-related disturbances trigger fish kills. In shallow lakes, high-velocity winds can break the thermal stratification and cause a turnover. Cold, heavy rainfall following prolonged hot weather or a severe hailstorm can also cause a summer turnover that brings bottom-residing anoxic water and decaying organic materials into the total water column. Oxygen depletion can result. Typical signs are low dissolved oxygen, decaying organic matter, foul odor, color change, and others, normally seen during oxygen depletion.

X2.1.3.3 Severe weather can cause disturbance of thermal stratification which can release large quantities of hydrogen sulfide (H_2S). High dissolved H_2S, even in the presence of adequate dissolved oxygen, can cause a "brown blood" condition and mortalities in fish. Environmental signs include an odor of H_2S in the water downwind from the site, black, decaying organic matter on the windward shore, disoriented, dying fish, and fish with dark, chocolate-colored gill filaments.

X2.1.3.4 In areas where manganese is abundant in soils of the watershed, dissolved manganous oxide may accumulate in the anoxic, acid hypolimnion to levels that are toxic to fish. Generally, because no fish are in the anoxic zone, the potential hazard usually goes unrecognized. However, if the stratification is disturbed (for example, by a cold rain, a turnover, or an internal seiche), a fish kill may occur.

X2.1.3.5 *Gas Supersaturation*—The solubility of gases in water is inversely related to temperature and directly related to atmospheric and hydrostatic pressures. Fish can suffer from gas bubble disease or gas bubble trauma. Obvious gas bubbles develop in the fins, under the skin, or around the eyes. With magnification, bubbles can be seen in the capillaries of the gills. An excellent discussion of problems caused by gas supersaturation in water was published by Marking **(39)**. Nitrogen supersaturation is usually involved in gas bubble disease, but oxygen supersaturation can also cause problems. If aquatic plants (such as the stonewort, *Chara sp.*) are abundant and weather conditions are ideal for photosynthesis, the plants may supersaturate the water with oxygen. If the water temperature rises or if the pressure changes, fish in the area may develop oxygen-related gas bubble disease.

X2.1.3.6 *Other Environmental Stressors*—Sometimes, an environmental stress may go unrecognized because no direct mortality occurred. Oxygen concentrations below 4 ppm, spawning, migrations, or elevated or depressed water temperatures may be significant stressors that reduce the resistance of fish to pathogens. For example, threadfin shad require warm water. If the temperature falls to 10°C or lower, the fish become severely stressed and may die; the weakened survivors then frequently develop bacterial or fungal infections that result in a fish kill. Post-spawning fish also have reduced resistance to pathogens; it is not uncommon to observe significant numbers of dead fish in spring. Incidents of fall-spawning species may also occur. Such kills are usually restricted to adults of a single species, but multiple species may be affected, depending on the chronology of their spawning.

X2.2 *Wildlife Incidents:*

X2.2.1 *Chemical Agents*:

X2.2.1.1 *Cholinesterase Inhibiting Insecticides*—These are organophosphate and carbamate insecticides. Signs in poisoned animals vary. Birds sickened by organophosphate or carbamate insecticides show lacrimation (tears), salivation (drooling), constriction of eye pupils, loss of muscle control, and perhaps clenched feet with an inability to stand or perch. Birds are often found on their backs near the site of application with eyes open. Mammals that have been exposed to organophosphate or carbamate pesticides exhibit many of the same symptoms as birds. Additional symptoms in mammals may include vomiting, diarrhea, twitching of facial muscles (in advanced cases this twitching could be pronounced throughout the muscles of the body), a stiff-legged, sawhorse-like walk, frequent coughing, and extreme hyperexcitability sometimes accompanied by convulsions just prior to death. Not all of these symptoms are present in all poisoned animals.

X2.2.1.2 *Strychnine*—Strychnine, a rodenticide now only used legally for below ground applications, causes a convulsive death in about 10 to 30 min. It is occasionally used illegally and may cause death in protected mammals. Dead animals would be found close to the point of application. Signs of agonal, stressful death will be seen. Strychnine grain baits are dyed green. These green-dyed grain baits of rolled oats, barley, milo, and other components may be found in hamburger or other meat used illegally to kill coyotes or dogs, or in dough baits fed to feral waterfowl.

X2.2.1.3 *Anticoagulants*—Anticoagulant grain baits are rodenticides legally used to kill ground squirrels, commensal rodents, and rabbits. The mode of action is to cause slow internal bleeding and eventual death. Signs of poisoning include excessive or minimal signs of blood. Hemorrhage may be obvious with bleeding through external orifices such as the nose, mouth, ears, or anus. Consequently the roof of the mouth may be very pale because of the blood loss. Wrist, elbow, and knee joints may be reddened. In some cases, massive internal bleeding occurs and the body cavities are filled with free blood. The stomach may be distended with water from the animal's attempts to overcome dehydration from bleeding. The animal may be found next to water. Death usually takes several days to occur, and the animal may be seen sick. Anticoagulant grain baits are usually dyed blue. Ground squirrels may show a bluish cast through the skin from the dye. Hunter killed wild pigs and raccoons have been found with blue colored fat, suggesting ingestion of anticoagulants.

X2.2.1.4 *Fumigants*—Fumigants are non-selective toxic gases most often used to control burrow-dwelling rodents. The gasses are either the result of combustion of solid materials, often in a cartridge, placed into a burrow, or volatilization of a liquid sprayed into the burrow. Very seldom are dead organisms found on the surface. Most animals die in the burrows. If exposure to fumigants is suspected, it may be necessary to excavate an entire burrow system to look for carcasses. This can be a lengthy and exhausting activity.

X2.2.1.5 *Other Pesticides*—Other pesticides that have been reported to cause wildlife mortality in Europe include a narcotic known as Alphachlorase; a molluscicide known as Metaldehyde, and pyrethroids, and herbicides known as paraquat, diquat, monochloroacetate.[8]

X2.2.1.6 *Ethylene Glycol*—Ethylene glycol (antifreeze) is the engine coolant commonly found in motor vehicles. Its sweet taste makes ethylene glycol very attractive to most mammals, and it can also prove attractive to birds. Cats are particularly susceptible to the toxic effects of this material. This material can often be found illegally disposed of in rural areas which border on urban development. The use of ethylene glycol has recently been used as a means of killing unwanted pest animals. Its use as a pesticide is illegal. A bowl or container of the material, with meat scraps or grain added as an attractant may be placed in an area frequented by nuisance animals. The victim of ethylene glycol poisoning often gives the appearance of intoxication. Death results from renal (kidney) failure. If exposure to ethylene glycol is suspected, be sure to collect at least the kidneys from the carcass because these are essential in demonstrating exposure.

X2.2.1.7 *Cyanide*—Cyanide is used extensively in the mining industry to extract precious metals from raw ore. A solution containing cyanide is repeatedly passed through piles of crushed ore (heap leaching). This solution is then processed to extract the metals and the remaining solution often is placed in outside impoundments. These ponds attract wading birds and waterfowl which become exposed to the cyanide and die.

X2.2.1.8 *Trace-Metals*—Trace elements can also be responsible for wildlife incidents. Trace elements can be mobilized in the environment as a result of mining or agricultural activities. Selenium is a trace metal that has been cited as the causative agent in several well-publicized cases of waterfowl and wading bird nesting failure in the Western United States. The selenium is deposited in the eggs of the birds and causes severe deformities in the chicks **(40)**. Problems with this element are often associated with areas which were once ocean sediments.

X2.2.1.9 Mercury toxicosis will occasionally occur in wildlife, particularly in grebes. Symptoms of organomercury poisoning will include evidence of central nervous system degeneration. Inorganic mercury poisoning will characteristically destroy the digestive tract resulting in death shortly after exposure.

X2.2.1.10 Lead poisoning is most commonly observed in birds, particularly waterfowl. With new hunting regulations which ban the use of lead shot in wetlands this problem has been significantly reduced. Ingestion of lead shot by scavenging raptors feeding on hunter killed carcasses has been reported for eagles, red-tailed hawks, and vultures. Vultures in particular appear to be very susceptible to poisoning by small quantities of lead shot or bullet fragments.

X2.2.2 *Biological Agents*:

X2.2.2.1 *Migratory Stress*—Some birds and mammal species migrate great distances during the course of a year. During these migrations, animals may die due to physical stresses of the migration. Adverse weather conditions or low food supplies during the time migratory herds or flocks arrive may result in large die-offs of weakened animals.

X2.2.2.2 *Population Cycles*—Population cycles commonly occur with small rodent species. The classic example of the mass die-offs of lemmings is well known. Other species such as voles (*Microtus* sp) also exhibit this population buildup and crash pattern.

X2.2.2.3 *Toxic Algae*—Certain algae and dinoflagellates can release toxins which will kill birds or mammals if they consume water containing these toxins. Deer and pronghorn antelope have been found dead around water holes where blooms of the cyanobacteria, *Microcystis aeruginosa*, have occurred. The toxins produced by this, and other genera of, cyanobacteria can act as anti-cholinesterase agents. Symptoms of exposure may be very similar to those in animals exposed to organophosphate or carbamate pesticides. Red tides often produce a toxin (domoic acid) that has been identified as the causative agent in some seabird kills **(41)**. Many native species of plants also produce toxins which may result in wildlife poisonings. Though not a common occurrence, it should not be discounted when investigating incidents involving any of the large ungulates **(16)**.

X2.2.2.4 *Diseases*—Because of the wide range of diseases which can affect wildlife and the great differences in susceptibility to a specific disease among species, a detailed discussion of diseases is best dealt with in other texts dedicated to that topic **(3,4,16)**.

X2.2.2.5 Because of the complexity of many diseases and the fact that disease symptoms can often mimic symptoms characteristic of exposure to pesticides or other toxins, the field investigator should try to work closely with a veterinarian whenever possible. Typically, the field investigator will not have the equipment readily at hand to conduct necropsies in the field in order to determine the cause of a wildlife loss. Wherever possible, entire carcasses should be collected and brought back to a laboratory setting where proper sterile procedures can be followed. This is very important, particularly when dealing with mammal die-offs, because some diseases can be transmitted to humans. If carcasses are too large to remove to a laboratory they should be iced in place if possible and appropriate personnel and equipment brought to the scene.

X2.2.2.6 *Viral Agents*—Viral agents have been identified as a causative factor in mammalian and avian die-offs. In mammals, hemorrhagic disease in deer and sheep can be caused by either of two viruses, epizootic hemorrhagic disease (EHD) virus, or blue-tongue (BT) virus. A third form of hemorrhagic disease caused by an adenovirus, has recently been identified from black-tail deer in California. Duck virus enteritis (DVE) or duck plague is an acute disease caused by a herpesvirus and is found only in waterfowl. Almost without fail domestic reared or feral (muscovy/city park) ducks are involved in outbreaks of this disease. External signs include convulsions, inability to fly, and in males, a prolapsed penis is often observed. These symptoms can be mistaken for indications of pesticide poisoning. Avian pox, caused by an avipoxvirus affects many species of birds causing the formation of large warty growths on non-feathered skin areas. Generally not fatal, the growths can become secondarily infected or can interfere with feeding, sight, and respiration. Outbreaks of the disease can occur in areas when populations of the principle vectors, mosquitos, dramatically increase.

X2.2.2.7 *Fungal Agents*—Fungal agents have not been reported as a causative factor in mammal die-offs. However, major bird die-offs have occurred occasionally as a result of aspergillosis. Caused by fungi of the genus *Aspergillus*, this disease is considered to be noncontagious and is often seen in hunter-crippled waterfowl or birds in crowded, confined conditions such as rehabilitation centers or blackbird roosts.

X2.2.2.8 *Bacterial Agents*—Two of the primary causes for massive die-offs of waterfowl are attributed to bacterial agents. Avian cholera is caused by *Pasteurella multocida*. Incidents involving hundreds to thousands of birds at one time occur annually in California, Southern Oregon, the Texas Panhandle, and the Rainwater Basin in Nebraska. Cholera outbreaks have also been reported for different species of gulls and for crows. Avian botulism is caused by ingestion of a toxin produced by *Clostridium botulinum*. This disease is responsible for large-scale die-offs of waterfowl and shore birds. The disease is typically spread by live birds ingesting fly maggots from dead fish or water bird carcasses, the maggots contain toxin produced by the bacteria in the rotting carcasses. Outbreaks of salmonellosis can also occur in wild bird populations. Caused by bacteria of the genus *Salmonella*, large-scale die-offs of passerine birds can occur. This is particularly evident in species (pine siskins, house sparrows, Northern orioles) that frequent back yard feeding stations. In 1993, a die-off of pine siskins occurred over an area extending from British Columbia Canada to the San Francisco Bay area of California. In mammals, *Francisella tularensis* is the bacteria responsible for tularemia. This disease is most commonly associated with die-offs of cottontail rabbits, jack rabbits, muskrats, and beavers. Infrequent large scale kills of rodents, primarily ground squirrels, rats, and mice can occur as a result of the bacterium *Yersinia pestis* that causes the disease known as plague.

X2.2.2.9 *Parasitic Agents*—Trichomoniasis in doves and pigeons is caused by a parasitic protozoan. The infected birds often contaminate food or water sources in back yard feeding stations. Other doves then ingest the contaminated food or water and become infected. Typically, only small localized incidents involving columbiform species occur from this disease but occasional large die-offs have been reported. Parasitic organisms (for example, tapeworms and round worms) are fairly common in mammals. They generally are not directly responsible for animal deaths and large-scale die-offs have not been reported in the past.

X2.2.3 *Physical Agents*:

X2.2.3.1 *Weather*—Lightning has been identified in several incidents involving waterfowl. The birds may be struck while in flight as part of a flock. Large numbers of carcasses may be scattered over a large land area, including the roofs of buildings in the area. These animals may have a burned odor with a streak of singed feathers starting on one wingtip and passing straight down the wing to the body. Often the burn streak will be visible on various internal organs during necropsy. Sudden, unseasonal, changes in weather conditions, such as cold fronts, can adversely affect populations **(17)**, causing localized die-offs.

X2.2.3.2 *Food Availability*—Because of fluctuations (natural or man-made) in prey populations, predator populations may experience noticeable fluctuations. If the prey base drops dramatically during nesting, owls, hawks, and their offspring may die due to starvation. Human disruptions of food supplies can also cause localized die-offs. Construction in an area can significantly impact the availability of browse for herbivores, resulting in starvation of weaker animals (older adults and juveniles) which are unable to compete for remaining food supplies.

X2.2.3.3 *Accidents*—The presence of man-made structures sometimes comes into conflict with wildlife, particularly birds. Along both coasts, migrating birds may hit the guy-wires that support marine radio antennas. When flocks of birds are migrating at night, large numbers of dead may be found in the morning on the ground throughout the area near these antennae. Characteristically they will have at least one broken wing, in some cases other physical injuries will be observed. Similar incidents can occur when high-voltage power transmission lines cross large areas of waterfowl habitat or waterfowl migration corridors. In areas where wind-powered electrical generators are present, birds may be struck by the rotating blades of the turbines. In Europe, large flocks of starlings have been reported flying into roads mistaking the roads for a different medium. Many birds die as a result of hitting panes of glass, not seeing a barrier.

X2.2.3.4 Hawks and eagles tend to hunt from, and nest on, elevated structures, often in open areas. Power poles are often utilized for these purposes. Species with large wing spans can make contact between two power lines and be electrocuted. The birds will typically have a burned odor and burns will be observable on the wings or feet.

X3. ENVIRONMENTAL SAMPLING GEAR FOR FISH AND WILDLIFE INCIDENTS

X3.1 *Camera or VCR*—Use the camera to document habitat conditions, affected fish or wildlife and their location. Photos or slides may be useful to the diagnostician or for evidence in a legal case; the use of a scale measure for close-up photographs is helpful.

X3.2 In some instances liquid nitrogen may be required as a biological agent.

X3.3 *Surgical Mask*—To protect against inhaling aerosol pathogens. A surgical mask will not protect against chemical fumes.

X3.4 *Plastic Bags (Large and Small)*—For collecting specimens of fish, wildlife, and plants. Double two appropriately sized bags, invert them, and put your hand into the bags and grasp the sample to be collected. Invert the bags again, pulling the sample through to the bag's bottom and seal the bags. It is recommended that all fish and wildlife analyzed for pesticides that they first be wrapped in aluminum foil prior to being placed in a plastic bag; do not use foil for specimens analyzed for trace-metals.

X3.5 *Duct Tape*—For sealing coolers or securing suspected pesticide-contaminated tissue samples to the outside area of a vehicle, and for sealing sleeves and or pant cuffs.

X3.6 *Chemically Clean Bottle(s)*—For collecting liquids and sediments suspected to contain contaminants (amber bottles for water and sediments to be used for residue analysis).

X3.7 *Hand Spade*—To collect sediment or dirt samples.

X3.8 *Betadine Surgical Scrub Soap or Bleach*—Wash your hands thoroughly (preferably more than once) after removing your surgical gloves.

X3.9 *Plastic Trash Bag(s)*—Place gloves and used plastic sample bag(s) into the trash bag and dispose trash at an approved trash collection facility.

X3.10 *String Tags*—Attach tag to the animal. Information on the tag should include location and date collected, investigation number, and collector's name.

X3.11 Labels for all sample jars and clear tape to attach labels to jars. Tags used on specimens should be indestructible, and the information cards should be placed in the specimen bag or container.

X3.12 Evidence tape to seal all samples as part of the chain of custody record.

X3.13 Blood vials and syringes for collecting samples if animals are sick but not dead.

X3.14 Flags to note locations of dead animals during initial site survey.

X3.15 *Indelible "Magic Marker" or "Sharpie Pen"*—To label tags and bags.

X3.16 *Insulated Cooler*—To transport specimens and samples.

X3.17 *Pocket Thermometer*—To measure water and air temperature.

X3.18 *Frozen "Blue Ice" Packs*—To cool specimens during transport.

X3.19 *Electronic Meters and Equipment*—To measure dissolved oxygen, pH, ammonia, chlorine, and other potential parameters which affect the environment.

X3.20 Aluminum foil to wrap all specimens for organic analyses. Do not use for specimens analyzed for trace-metals.

X3.21 Data sheets for collecting information.

X4. CHAIN OF CUSTODY FORM

SAMPLER / PHONE #	SEND RESULTS TO	FOR LABORATORY USE ONLY
ADDRESS	ADDRESS	LAB NUMBER
CITY / ZIP	CITY / ZIP	FIELD NUMBER
		LAB STORAGE
DATE REQUIRED/REASON	COPIES TO	SPILL TITLE
SHIPPED VIA	ADDRESS	SUSPECT
	CITY / STATE / ZIP	INDEX-PCA

❑ Fish & Wildlife Loss Date:__________ Region: __________

❑ Suspected or Potential Problem

❑ Routine Analysis

ANALYSIS REQUESTED →

Water Temp: °F or °C pH: DO: mg/L Conductivity: umhos/cm

Sample Identification/Location (Draw Map on Separate Sheet If Necessary)	Collection		PETROLEUM FINGERPRINT	TRACE ELEMENTS (SPECIFY BELOW)	PESTICIDES (SPECIFY BELOW)								Sample Type					Number of Containers				Preservation	
	Date	Time											WATER	FILTERED WATER	SOIL	TISSUE		PLASTIC	GLASS	VOA VIAL		TEMP	ACID

Problem Description

Suspect/Incident Location

Comments/Special Instructions

SAMPLE(S) RELINQUISHED BY (SIGNATURE)	PRINT NAME	DATE	RECEIVED BY (SIGNATURE)	PRINT NAME

WHITE, CANARY, PINK -- LAB COPIES
GOLDENROD - SUBMITTER

FIG. X4.1 Chain of Custody Form

X5. STATE AGENCIES RESPONSIBLE FOR INVESTIGATION OF INCIDENTS

STATE ALABAMA
AGENCY STATE EXTENSION SERVICE
ADDRESS AUBURN UNIVERSITY
CITY AUBURN
ZIP 36849
PHONE 205-844-9233
FAX 205-844-9234

STATE ALABAMA
AGENCY DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
ADDRESS 1751 CONGRESSMAN DICKENS DRIVE
CITY MONTGOMERY
ZIP 36109
PHONE 205-260-2700
FAX 205-272-8131

STATE ARIZONA
AGENCY GAME AND FISH DEPARTMENT
ADDRESS 2222 W. GREENWAY RD.
CITY PHOENIX
ZIP 85023-4312
PHONE 602-789-3375
FAX 602-789-3920

STATE ARIZONA
AGENCY DEPARTMENT OF ENVIRONMENTAL QUALITY
ADDRESS 3033 N. CENTRAL
CITY PHOENIX
ZIP 85012
PHONE 602-207-4545
FAX 602-207-4528

STATE ARKANSAS
AGENCY STATE PLANT BOARD/PESTICIDE DIVISION
ADDRESS P.O. BOX 1069
CITY LITTLE ROCK
ZIP 72203
PHONE 501-225-1598
FAX 501-225-3590

STATE ARKANSAS
AGENCY DEPARTMENT OF POLLUTION CONTROL AND ECOLOGY
ADDRESS P.O. BOX 8913
CITY LITTLE ROCK
ZIP 72219-8913
PHONE 501-562-7444
FAX 501-562-4632

STATE ALASKA
AGENCY DEPARTMENT OF ENVIRONMENTAL CONSERVATION
ADDRESS 500 SOUTH ALASKA ST.
CITY PALMER
ZIP 99645
PHONE 907-745-3236
FAX 907-745-8125

STATE CALIFORNIA
AGENCY DEPARTMENT OF FISH AND GAME
ADDRESS PESTICIDE INVESTIGATIONS UNIT
1701 NIMBUS ROAD, SUITE F
CITY RANCHO CORDOVA
ZIP 95670
PHONE 916-358-2950
FAX 916-358-2953

STATE CALIFORNIA
AGENCY DEPARTMENT OF PESTICIDE REGULATION
ADDRESS PESTICIDE ENFORCEMENT BRANCH
1020 N STREET
CITY SACRAMENTO
ZIP 95812
PHONE 916-445-3920
FAX 916-445-3907

STATE COLORADO
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS 317 W. PROSPECT
CITY FORT COLLINS
ZIP 80526
PHONE 970-484-2937
FAX 303-490-2621

STATE CONNECTICUT
AGENCY DEPARTMENT OF ENVIRONMENTAL PROTECTION
ADDRESS PESTICIDE MANAGEMENT DIVISION
STATE OFFICE BUILDING
CITY HARTFORD
ZIP 06106
PHONE 203-566-5148
FAX 203-566-4924

STATE DELAWARE
AGENCY DIVISION OF FISH AND WILDLIFE
ADDRESS DEPARTMENT OF NATURAL RESOURCES
P.O. BOX 1401
CITY DOVER
ZIP 19903
PHONE 302-739-3441
FAX 302-739-6157

STATE DISTRICT OF COLUMBIA
AGENCY REGULATION ADMINISTRATION
ADDRESS PESTICIDE BRANCH-ROOM 203
2100 MARTIN LUTHER KING, JR. AVE. SE
CITY WASHINGTON
ZIP 20020
PHONE 202-404-1136
FAX 202-404-1141

STATE FLORIDA
AGENCY DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES
ADDRESS COMPLIANCE OFFICE
3125 CONNER BLVD., MC-1
CITY TALLAHASSEE
ZIP 32399-1650
PHONE 904-488-3314
FAX 904-922-2134

STATE FLORIDA
AGENCY GAME AND FRESHWATER FISH COMMISSION
ADDRESS DIVISION OF WILDLIFE
620 SOUTH MERIDIAN ST.
CITY TALLAHASSEE
ZIP 32399-1600
PHONE 904-488-3831
FAX 904-488-6988

STATE GEORGIA
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS ENTOMOLOGY AND PESTICIDE DIVISION
19 MARTIN LUTHER KING, JR. DR., RM. 550
CITY ATLANTA
ZIP 30334
PHONE 404-656-3645
FAX 404-656-9380

STATE GEORGIA
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS AGRICULTURE BLDG.
CAPITOL SQUARE
CITY ATLANTA
ZIP 30334-2001
PHONE 404-651-7861
FAX

STATE HAWAII
AGENCY DEPARTMENT OF LAND AND NATURAL RESOURCES
ADDRESS DIVISION OF AQUATIC RESOURCES
1151 PUNCHBOWL, ROOM 330
CITY HONOLULU
ZIP HI
PHONE 808-587-0111
FAX 808-587-0115

STATE IDAHO
AGENCY FISH AND GAME DEPARTMENT
ADDRESS P.O. BOX 25
CITY BOISE

ZIP	83707
PHONE	208-334-2920
FAX	208-334-2114
STATE	ILLINOIS
AGENCY	ENVIRONMENTAL PROTECTION/BUREAU OF WATER
ADDRESS	2200 CHURCHILL ROAD, PO BOX 19276
CITY	SPRINGFIELD
ZIP	62794-9276
PHONE	217-782-1696
FAX	217-782-9891
STATE	ILLINOIS
AGENCY	ILLINOIS NATURAL HISTORY SURVEY
ADDRESS	CENTER FOR WILDLIFE ECOLOGY 607 EAST PEABODY
CITY	CHAMPAIGN
ZIP	61820
PHONE	217-333-5199
FAX	217-333-4949
STATE	ILLINOIS
AGENCY	DEPARTMENT OF CONSERVATION
ADDRESS	DIVISION OF HERITAGE 524 SOUTH 2ND STREET
CITY	SPRINGFIELD
ZIP	62701
PHONE	217-785-8774
FAX	217-785-8277
STATE	INDIANA
AGENCY	STATE CHEMIST OFFICE/PESTICIDE DIVISION
ADDRESS	1154 BIOCHEMISTRY BUILDING PURDUE UNIVERSITY
CITY	WEST LAFAYETTE
ZIP	47907-1154
PHONE	317-494-1589
FAX	317-494-4331
STATE	INDIANA
AGENCY	DEPARTMENT OF NATURAL RESOURCES
ADDRESS	DIVISION OF FISH AND WILDLIFE 402 WEST WASHINGTON RM. W273
CITY	INDIANAPOLIS
ZIP	46204
PHONE	317-232-4080
FAX	317-232-8150
STATE	IOWA
AGENCY	DEPARTMENT OF NATURAL RESOURCES
ADDRESS	900 EAST GRANT
CITY	DES MOINES
ZIP	50319
PHONE	515-281-6614
FAX	515-281-8895
STATE	KANSAS
AGENCY	DEPARTMENT OF HEALTH AND ENVIRONMENT
ADDRESS	OFFICE OF SCIENCE AND SUPPORT FORBES FIELD BLD. 740
CITY	TOPEKA
ZIP	66620
PHONE	913-296-1522
FAX	913-296-6247
STATE	KANSAS
AGENCY	DEPARTMENT OF WILDLIFE AND PARKS
ADDRESS	ENVIRONMENTAL SERVICES SECTION RURAL RT.2/BOX 54A
CITY	PRATT
ZIP	67124
PHONE	316-672-5911
FAX	316-672-6020
STATE	KENTUCKY
AGENCY	DEPARTMENT OF FISH AND WILDLIFE
ADDRESS	#1 GAME FARM RD.
CITY	FRANKFORT
ZIP	40601
PHONE	502-564-5448
FAX	502-564-6508
STATE	LOUISIANA
AGENCY	DEPARTMENT OF AGRICULTURE AND FORESTRY
ADDRESS	PESTICIDE DIVISION P.O. BOX 3596
CITY	BATON ROUGE
ZIP	70821-3596
PHONE	504-4925-3690
FAX	504-925-3760
STATE	MAINE
AGENCY	DEPARTMENT OF AGRICULTURE, FOOD AND RURAL
ADDRESS	MAINE BOARD OF PESTICIDE CONTROL STATE HOUSE STATION #28
CITY	AUGUSTA
ZIP	04333
PHONE	207-287-2731
FAX	207-287-7548
STATE	MARYLAND
AGENCY	DEPARTMENT OF NATURAL RESOURCES
ADDRESS	P.O. BOX 68
CITY	WYE MILLS
ZIP	21679
PHONE	410-827-8612
FAX	401-827-5186
STATE	MARYLAND
AGENCY	DEPARTMENT OF AGRICULTURE
ADDRESS	50 HARRY S. TRUMAN PARKWAY
CITY	ANNAPOLIS
ZIP	21401
PHONE	410-841-5710
FAX	410-841-2765
STATE	MASSACHUSETTS
AGENCY	DEPARTMENT OF MARINE FISHERIES
ADDRESS	100 CAMBRIDGE STREET
CITY	BOSTON
ZIP	02202
PHONE	617-727-3193
FAX	617-727-7988
STATE	MASSACHUTTS
AGENCY	DEPARTMENT OF ENVIRONMENTAL PROTECTION
ADDRESS	OFFICE OF WATER MANAGEMENT P.O. BOX 116
CITY	NORTH GRAFTON
ZIP	01536
PHONE	508-839-3469
FAX	508-839-3469
STATE	MASSACHUSETTS
AGENCY	DEPARTMENT OF FOOD AND AGRICULTURE
ADDRESS	PESTICIDE BUREAU 100 CAMBRIDGE STREET
CITY	BOSTON
ZIP	02202
PHONE	617-727-3020
FAX	617-727-7235
STATE	MICHIGAN
AGENCY	DEPARTMENT OF NATURAL RESOURCES
ADDRESS	ROSE LAKE WILDLIFE DISEASE LAB 8562 EAST STOLL RD.
CITY	EAST LANSING
ZIP	48823
PHONE	517-373-9358
FAX	517-641-6663
STATE	MICHIGAN
AGENCY	DEPARTMENT OF AGRICULTURE
ADDRESS	P.O. BOX 30017
CITY	LANSING
ZIP	48909
PHONE	517-335-6838
FAX	517-335-4540
STATE	MINNESOTA
AGENCY	DEPARTMENT OF NATURAL RESOURCES
ADDRESS	ECOLOGICAL SERVICES SECTION P.O. BOX 25, 500 LAFAYETTE
CITY	ST. PAUL
ZIP	55155-4025
PHONE	612-296-0782

FAX 612-296-1811

STATE MISSISSIPPI
AGENCY DEPARTMENT OF ENVIRONMENTAL QUALITY
ADDRESS OFFICE OF POLLUTION CONTROL
CITY PEARL
ZIP 39208
PHONE 601-961-5183
FAX 601-939-5137

STATE MISSOURI
AGENCY DEPARTMENT OF CONSERVATION
ADDRESS 1110 SOUTH COLLEGE AVE.
CITY COLUMBIA
ZIP 65201
PHONE 314-882-9880
FAX 314-874-8849

STATE MONTANA
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS AGRICULTURE/LIVESTOCK BUILDING, CAPITOL STATION
CITY HELENA
ZIP 59620-0201
PHONE 406-444-3140
FAX 406-444-5409

STATE NEBRASKA
AGENCY DEPARTMENT OF ENVIRONMENTAL QUALITY
ADDRESS P.O. BOX 98922
CITY LINCOLN
ZIP 68509-8922
PHONE 402-471-2186
FAX 402-471-2909

STATE NEVADA
AGENCY DEPARTMENT OF WILDLIFE
ADDRESS 1375 MOUNTAIN CITY HIGHWAY
CITY ELKO
ZIP 89801
PHONE 702-738-5332
FAX 702-738-2485

STATE NEVADA
AGENCY STATE DEPARTMENT OF WILDLIFE
ADDRESS P.O. BOX 10678
CITY RENO
ZIP 89520
PHONE 702-688-1530
FAX 702-688-1595

STATE NEW YORK
AGENCY DEPARTMENT ENVIRONMENTAL CONSERVATION
ADDRESS HALE CREEK FIELD STATION
7235 STEELE AVE. EXTENSION
CITY GLOVERSVILLE
ZIP 12078
PHONE 518-773-7318
FAX 518-773-7319

STATE NEW YORK
AGENCY DEPARTMENT OF ENVIRONMENTAL CONSERVATION
ADDRESS 50 WOLF ROAD
DIVISION OF FISH AND WILDLIFE
CITY ALBANY
ZIP 12233
PHONE 518-439-8017
FAX 518-475-1587

STATE NEW HAMPSHIRE
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS DIVISION OF PESTICIDE CONTROL
P.O. BOX 2042
CITY CONCORD
ZIP 03302-2042
PHONE 603-271-3550
FAX 603-271-1109

STATE NEW MEXICO
AGENCY DEPARTMENT OF GAME AND FISH
ADDRESS P.O. BOX 25112
CITY SANTA FE
ZIP 87504
PHONE 505-827-9910
FAX 505-827-9956

STATE NEW JERSEY
AGENCY DEPARTMENT OF ENVIRONMENTAL PROTECTION
ADDRESS FRESHWATER FISHERIES LAB
P.O. BOX 394
CITY LEBANON
ZIP 08833
PHONE 908-236-2118
FAX 908-236-7280

STATE NEW MEXICO
AGENCY DEPARTMENT OF GAME AND FISH
ADDRESS P.O. BOX 25112
CITY SANTA FE
ZIP 87504
PHONE 505-827-9910
FAX 505-827-9956

STATE NEW HAMPSHIRE
AGENCY DIVISION OF PUBLIC HEALTH SERVICE
ADDRESS 6 HAZEN DRIVE
CITY CONCORD
ZIP 03301
PHONE 603-271-4664
FAX 603-273-3745

STATE NEW JERSEY
AGENCY DEPARTMENT OF ENVIRONMENTAL PROTECTION
ADDRESS PESTICIDE CONTROL PROGRAMS
CN 411
CITY TRENTON
ZIP 08625
PHONE 609-530-4136
FAX 609-530-8324

STATE NORTH CAROLINA
AGENCY DEPARTMENT OF AGRICULTURE/PESTICIDE UNIT
ADDRESS P.O. BOX 27647
CITY RALEIGH
ZIP 27611
PHONE 919-733-3556
FAX 919-733-9796

STATE NORTH DAKOTA
AGENCY GAME AND FISH DEPARTMENT/WILDLIFE DIVISION
ADDRESS 100 N. BISMARCK EXPRESSWAY
CITY BISMARCK
ZIP 58501
PHONE 701-221-6340
FAX 701-221-6352

STATE OHIO
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS DIVISION OF WILDLIFE
1840 BELCHER DRIVE
CITY COLUMBUS
ZIP 43224-1329
PHONE 614-265-7093
FAX

STATE OKLAHOMA
AGENCY DEPARTMENT OF WILDLIFE CONSERVATION
ADDRESS NATURAL RESOURCES DIVISION
1801 NORTH LINCOLN BLVD.
CITY OKLAHOMA CITY
ZIP 73105-4998
PHONE 405-521-3851
FAX 405-521-6535

STATE OREGON
AGENCY DEPARTMENT OF FISH AND WILDLIFE
ADDRESS HABITAT CONSERVATION DIVISION
P.O. BOX 59
CITY PORTLAND
ZIP 97202
PHONE 503-229-6967
FAX 503-229-5969

STATE OREGON
AGENCY DEPARTMENT OF STATE POLICE
ADDRESS FISH AND WILDLIFE DIVISION
400 PUBLIC SERVICE BUILDING

CITY SALEM
ZIP 97310
PHONE 503-378-3720
FAX 503-363-5475

STATE PENNSYLVANIA
AGENCY FISH AND BOAT COMMISSION
ADDRESS P.O. BOX 67000
CITY HARRISBURG
ZIP 17106
PHONE 717-657-4542
FAX 717-657-4549

STATE RHODE ISLAND
AGENCY DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
ADDRESS DIVISION OF AGRICULTURE
22 HAYES STREET
CITY PROVIDENCE
ZIP 02908
PHONE 401-227-2781
FAX 401-228-6047

STATE RHODE ISLAND
AGENCY DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
ADDRESS DIVISION OF WATER RESOURCES
291 PROMINADE STREET
CITY PROVIDENCE
ZIP 02098
PHONE 401-277-6519
FAX 401-521-4230

STATE SOUTH CAROLINA
AGENCY DEPARTMENT OF HEALTH AND ENVIRONMENTAL CONTROL
ADDRESS EMERGENCY RESPONSE SECTION
2600 BULL ST.
CITY COLUMBIA
ZIP 29201
PHONE 803-935-7302
FAX 803-935-6322

STATE SOUTH DAKOTA
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS ANDERSON BUILDING
445 EAST CAPITOL
CITY PIERRE
ZIP 57510-3185
PHONE 605-773-3375
FAX 605-773-3481

STATE TENNESSEE
AGENCY WILDLIFE RESOURCES AGENCY
ADDRESS P.O. BOX 40747
CITY NASHVILLE
ZIP 37204
PHONE 615-781-6500
FAX 615-741-4606

STATE TENNESSEE
AGENCY DEPARTMENT OF CONSERVATION
ADDRESS DIVISION OF WATER POLLUTION CONTROL
6TH FLOOR, L AND C ANNEX/401 CHURCH ST.
CITY NASHVILLE
ZIP 37243-1534
PHONE 615-532-0625
FAX 615-532-0046

STATE TEXAS
AGENCY NATURAL RESOURCE CONSERVATION COMMISSION
ADDRESS FIELD OPERATIONS DIVISION
P.O. BOX 13087, MC174
CITY AUSTIN
ZIP 78711-3087
PHONE 512-239-0400
FAX 512-239-0404

STATE TEXAS
AGENCY TEXAS PARKS AND WILDLIFE DEPARTMENT
ADDRESS FRESHWATER CONSERVATION BRANCH
4200 SMITH SCHOOL ROAD
CITY AUSTIN
ZIP 78744
PHONE 512-389-4726
FAX 512-389-4799

STATE UTAH
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS DIVISION OF WILDLIFE RESOURCES
1596 WEST NORTH TEMPLE
CITY SALT LAKE CITY
ZIP 84116-3195
PHONE 801-538-4700
FAX 801-538-4709

STATE VERMONT
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS 116 STATE STREET
CITY MONTPELIER
ZIP 05620-2901
PHONE 802-828-2431
FAX 802-828-2361

STATE VIRGINIA
AGENCY DEPARTMENT OF GAME AND INLAND FISHERIES
ADDRESS P.O. BOX 11104
CITY RICHMOND
ZIP 23230-1104
PHONE 804-367-1000
FAX 804-367-9147

STATE WASHINGTON
AGENCY DEPARTMENT OF AGRICULTURE
ADDRESS PESTICIDE MANAGEMENT DIVISION/
COMPLIANCE SECTION
P.O. BOX 42589
CITY OLYMPIA
ZIP 98504-2589
PHONE 206-902-2036
FAX 206-902-2093

STATE WASHINGTON
AGENCY DEPARTMENT OF FISH AND WILDLIFE
ADDRESS WILDLIFE MANAGEMENT SECTION
600 CAPITOL WAY, N.
CITY OLYMPIA
ZIP 98501-1091
PHONE 206-902-2661
FAX 206-664-0912

STATE WEST VIRGINIA
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS 1900 KANAWHA BLVD EAST
CITY CHARLESTON
ZIP 25305
PHONE 304-558-2771
FAX 304-558-3147

STATE WEST VIRGINIA
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS P.O. BOX 67
CITY ELKINS
ZIP 26241
PHONE 304-637-0245
FAX 304-637-0250

STATE WISCONSIN
AGENCY DEPARTMENT OF NATURAL RESOURCES
ADDRESS P.O. BOX 7921
CITY MADISON
ZIP 53707
PHONE 608-266-8839
FAX 608-266-3696

STATE WYOMING
AGENCY GAME AND FISH DEPARTMENT/FISHERIES DIV.
ADDRESS 5400 BISHOP BLVD.
CITY CHEYENNE
ZIP 82006
PHONE 307-777-4559
FAX 307-777-4610

STATE WYOMING
AGENCY GAME AND FISH DEPARTMENT
ADDRESS P.O. BOX 3312 UNIVERSITY STATION
CITY LARAMIE
ZIP 82071
PHONE 307-766-5629
FAX 307-766-5630

X6. FISH AND WILDLIFE FIELD INVESTIGATION FORM

Date of Loss [day | month | year]

Report [number | region | year]

Loss

____ Fish
____ Birds
____ Mammals
____ Other

Suspected Cause

____ Disease
____ Pesticide
____ Fertilizer
____ Pollution
____ Other (DO, Temp, etc.)
____ Unknown

In

____ Wild Populations
____ Installation

Loss Site

____ River or Stream
____ Lake or Pond
____ Estuary or Bay
____ Ocean
____ Land

Animal Loss Information

Species	Size Range	Approx. Number

Animal Types Involved

____ % Game
____ % Non-game
____
100% Total

Severity

____ Total
____ Heavy
____ Moderate
____ Light

Extent of Loss

____ Miles of Stream
____ Acres of Lake, Bay, or Land

Duration of Loss

____ Days
____ Hours

Town ____________ County ____________

Specific Location ____________

____________ T______ R______ S______

Loss First Reported By ____________
name address phone date

Additional Witnesses ____________
name address phone

Party believed responsible for loss ____________

Additional Forms Completed

____
____
____ U.S EPA (Form 7500-8)

Person Notified	Date
Dept. of Fish & Game ________	________
Dept. of Food & Ag ________	________
Reg. Water Qual. Control Bd. ________	________
County Ag. Comm. ________	________
Dept. of Health Serv. ________	________
Dept. of Industrial Safety ________	________
U.S. EPA ________	________
Other ________	________

FIG. X6.1 Fish and Wildlife Field Investigation Form

FACTUAL DIAGRAM/NARRATIVE

ALL MEASUREMENTS ARE APPROXIMATE AND NOT TO SCALE UNLESS STATED (SCALE=)

1″

2″

3″

4″

INDICATE NORTH

1″ 2″ 3″ 4″ 5″ 6″ 7″ 8″

NARRATIVE:

PREPARED BY	PREPARED	REVIEWED/APPROVED BY	APPROVED
NAME/TITLE	MO DAY YR	NAME/TITLE	MO DAY YR

FIG. X6.1 Fish and Wildlife Field Investigation Form *(continued)*

REFERENCES

(**1**) Urban, D., and Cook, N., *Standard Evaluation Procedure: Ecological Risk Assessment*, EPA 540/9-85-001, U.S. Environmental Protection Agency, Washington, DC, 1986.

(**2**) Fisher, L., *Decisions on Ecological Fate and Effects Task Force*, U.S. Environmental Protection Agency, Memorandum, Washington, DC, Oct. 28, 1992.

(**3**) Davidson, W. R., and Nettles, V. F., *Field Manual of Wildlife Diseases in the Southeastern United States*, Southeastern Cooperative Wildlife Disease Study, Athens, GA, 1988.

(**4**) Friend, M., ed., *Field Guide to Wildlife Diseases*, U.S. Fish and Wildlife Service Resource Publication 167, 1987.

(**5**) Meyer, F. P., and Barclay, L. A., *Field Manual for the Investigation of Fish Kills*, U.S. Fish and Wildlife Service Resource Publication 177, Washington, DC, 1990.

(**6**) International Technical Information Institute, *Toxic and Hazardous Industrial Chemicals Safety Manual*, Tokyo, Japan, 1977; Sax, N. I., *Dangerous Properties of Industrial Materials*, 6th ed., Van Nostrand Reinhold Co., New York, NY, 1984; Patty, F. A., ed., *Industrial Hygiene and Toxicology*, Vol II, 2nd ed., Interscience, New York, NY, 1963; Hamilton, A., and Hardy, H. L., *Industrial Toxicology*, 3rd ed., Publishing Sciences Group, Inc., Acton, MA, 1974; Goselin, R. E., Hodge, H. C., Smith, R. P., and Gleason, M. N., *Clinical Toxicology of Commercial Products*, 4th ed., Wincidentiams and Wilkins Co., Baltimore, MD, 1976.

(**7**) Green, N. E., and Turk, A., *Safety in Working with Chemicals*, MacMillan, New York, NY, 1978; National Research Council, *Prudent Practices for Handling Hazardous Chemicals in Laboratories*, National Academy Press, Washington, DC, 1981; Waters, D. B., ed., *Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances*, Ann Arbor Science, Ann Arbor, MI, 1980; Fawcett, H. H., and Wood, W. S., eds., *Safety and Accident Prevention in Chemical Operations*, 2nd ed., Wiley-Interscience, New York, NY, 1982.

(**8**) National Council on Radiation Protection and Measurement, "Basic Radiation Protection Criteria," NCRP Report No. 39, Washington, DC, 1971; Shapiro, J., *Radiation Protection*, 2nd ed., Harvard University Press, Cambridge, MA, 1981.

(**9**) National Institutes of Health, NIH Guidelines for the Laboratory Use of Chemical Carcinogens, *NIH Publication No. 81-2385*, Bethesda, MD, May 1981.

(**10**) Thurston, R. V., Russo, R. C., Fetterolf, C. M., Jr., Edsall, T. A., and Barber, Y. M., Jr., eds., *A Review of the EPA Red Book: Quality Criteria for Water*, Water Quality Section, American Fisheries Society, Bethesda, MD, 1979.

(**11**) For example, Mayer, F. L., Jr., and Ellersieck, M. R., *Manual of Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 Species of Freshwater Animals*, U.S. Fish and Wildlife Service, Resource Publication 160, 1986; Hunn, J., *Field Assessment of the Effects of Contaminants on Fishes*, U.S. Fish and Wildlife Service, Biological Report 88(19), Washington, DC, 1988.

(**12**) Mayer, F. L., Jr., *Acute Toxicity Handbook of Chemicals to Estuarine Organisms*, EPA 600/8-87-017, U.S. EPA, Environmental Research Laboratory, Gulf Breeze, FL, 1987.

(**13**) Weed Science Society of America, *Herbicide Handbook*, 6th ed., Weed Science Society of America, Champaign, IL, 1989.

(**14**) Snieszko, S. F., "Remarks on Some Facets of Epizootiology of Bacterial Fish Diseases," *Developments in Industrial Microbiology*, Vol 5, 1964, pp. 97–100.

(**15**) Thorne, E. T., Kingston, N., Jolley, W. R., and Bergstrom, R. C., eds, *Diseases of Wildlife in Wyoming*, Wyoming Game and Fish Department, 1982.

(**16**) Littrell, E. E., "Swallow Mortality During The "March Miracle" in California," *Calif Fish and Game*, Vol 78(3), 1992, pp. 128–130.

(**17**) American Public Health Association, American-Water Works Association, and Water Pollution Control Federation, *Standard Methods for the Examination of Water and Wastewater*, 16th ed., Washington, DC, 1985.

(**18**) U.S. Environmental Protection Agency, *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*, 1979, EPA 600/4-79-019.

(**19**) For example, U.S. EPA, *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA 600/4-89/001, 1989, U.S. EPA, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, EPA 600/4-90/027, 1990.

(**20**) Hill, D. M., "Fish Kill Investigation Procedures," *Fisheries Techniques*, Nielsen, L. A., and Johnson, D. L., eds., American Fisheries Society, Bethesda, MD, 1983, pp. 261–274.

(**21**) Davis, W., *Fish Kill Investigation Procedures*, The Pollution Committee-Southern Division, American Fisheries Society, Bethesda, MD, 1986.

(**22**) For example, U.S. Department of Justice, *Guidelines for Narcotic and Dangerous Drug Evidence Handling and Security Procedures*, Drug Enforcement Administration and International Association of Chiefs of Police, no date, Washington, DC; U.S. Fish and Wildlife Service, *Law Enforcement Manual*, Washington, DC, 1982.

(**23**) U.S. EPA, *Handbook for Sampling and Sample Preservation of Water and Wastewater*, U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 1982, EPA-600/4-82-029.

(**24**) Weber, C. I., *Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents*, U.S. EPA, Washington, DC, EPA 670/4-73-001, 1973.

(**25**) Palmer, M., *Methods Manual for Bottom Sediment Sample Collection*, U.S. EPA, Great Lakes National Program Office, Chicago, IL, EPA 905/4-85-004, 1984.

(**26**) Tetra Tech, Inc., *Recommended Protocols for Measuring Conventional Sediment Variables in Puget Sound*, Puget Sound Estuary Program, Bellevue, WA, 1986.

(**27**) Morrison, J. K., and Smith, C. E., *The Fixation and Handling of Fish Tissues Intended for Histological Examination*, U.S. Fish and Wildlife Service, Bozeman Information Leaflet 21, 1981.

(**28**) Yasutake, W. T., *Collection and Preparation of Fish Specimens for Histological Examination*, U.S. Fish and Wildlife Service, Fish and Wildlife Leaflet 11, 1987.

(**29**) Canadian Council of Resource and Environment Ministers, *Canadian Water Quality Guidelines*, 1994.

(**30**) Sprague, J. B.," Factors that Modify Toxicity," *Fundamentals of Aquatic Toxicology*, Rand, G. M., and Petrocelli, S. R., eds., Hemisphere Publishing Corp., New York, NY, 1985.

(**31**) Hunn, J. B., "Role of Calcium in Gill Function in Freshwater Fishes," *Comparative Biochemistry and Physiology*, Vol 82A, 1985, pp. 543–547.

(**32**) Mance, G., *Pollution Threat of Heavy Metals in the Aquatic Environments*, Elsevier Science Publishing Co., New York, NY, 1987, p. 372.

(**33**) Hunn, J. B., and Allen, J. L., "Movement of Drugs Across the Gills of Fishes," *Annual Review of Pharmacology*, Vol 14, 1974, pp. 47–55.

(**34**) Spacie, A., and Hamelink, J. L.," Bioaccumulation," *Fundamentals of Aquatic Toxicology*, Rand, G. M., and Petrocelli, S. R., eds., Hemisphere Publishing Corp., New York, NY, 1985, pp. 495–525.

(**35**) *Selected Environmental Law Statutes 1995-1996*, Educational Edition, West Publishing Company, St. Paul, MN, 1995.

(**36**) Falconer, I., *Algal Toxins in Seafood and Drinking Water*, Academic Press, San Diego, CA, 1978.

(**37**) Lassus, P., Arzul, G., Erard-Le Denn, E., Gentien, P., Marcaillou-Le

Baut, C., eds., *Harmful Marine Algal Blooms* (*Proceedings of the Sixth International Conference of Toxic Marine Phytoplankton*, October 1993, Nates, France), Lavoisier, New York, 1995.

(**38**) Rensel, J. E., "Severe Blood Hypoxia of Atlantic Salmon (*Salmo Salar*) Exposed to Marine Diatom (*Chaetoceros concavicornis*) in Smayda," T. J., and Shimizu, eds., *Toxic Phytoplankton Blooms in the Sea*, Elsevier Publications, pp. 625–630.

(**39**) Marking, L. L., *Gas Supersaturation in Fisheries: Causes, Concerns, and Cures*, U.S. Fish and Wildlife Service, Fish and Wildlife Leaflet 9, 1987.

(**40**) Ohlendorf, H. M., Hoffman, D. J., Saiki, M. K., and Aldrich, T. W., "Embryonic Mortality and Abnormalities of Aquatic Birds: Apparent Impacts of Selenium from Irrigation Drainwater," *Science of the Total Environment*, Vol 52, 1986, pp. 49–63.

(**41**) Work, T. M., Beale, A. M., Fritz, L., Quilliam, M. A., Silver, M., Buck, K., and Wright, J. L. C., "Demoic Acid Intoxification of Brown Pelicans and Cormorants in Santa Cruz, California," *Toxic Phytoplankton Blooms in the Sea*, Elsevier Science Publishers, 1993, pp. 643–649.