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

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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:

	Section
Referenced Documents	2
Terminology	3
Significance and Use	4
Laws and Regulations	4.1
Benefits of Maintaining Records	4.2
Ecological Risk Databases	4.3
Ecological Risk Assessments	4.4
Precautionary Steps and Safety	5
Determining the Cause of the Incident	6
Common Causes of Fish Mortality	6.1
Common Causes of Wildlife Mortality	6.2
Investigation of Incidents	7
Quality Control and Standard Operating Procedures	7.1
Planning Field Investigation	7.2
Interagency Coordination	7.3
Chain of Custody	7.4
Comprehensive and Systematic Collection of Samples and Information	7.5
Analyses of Samples	7.6
Analyses of Data	7.7
Determining Significance of Investigation	7.8
Reporting of Incidents	8
Format of Report	8.1
Documentation of Information	8.2
Appendixes	
References	

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:*[2]

D4687 Guide for General Planning of Waste Sampling

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate

E1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians

E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes

E1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*

E1367 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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E1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates
E1463 Guide for Conducting Static and Flow-Through Acute Toxicity Tests With Mysids From the West Coast of the United States
E1525 Guide for Designing Biological Tests with Sediments
E1705 Terminology Relating to Biotechnology

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[3] 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

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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 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[3] 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**).[4] 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

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

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suspected of being, carcinogenic (**9**). Guide D4687 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.

5.5 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 D4687 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,[5] American Fisheries Society (**10**), U.S. Fish and Wildlife Service (**11**), Mayer (**12**), and Weed Science Society of America (**13**).

[5] 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.

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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 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

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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.[6] 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. See Note 1. It is more important to reference the materials and methods used in the investigation than to use any particular method.

NOTE 1—For example, see Guides E729, E1192, E1241, E1295, E1367, and E1463 and Terminology E1705.

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),

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

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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 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

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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

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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

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