assessment area is significantly less than mean values for a population at a control area, and ALAD depression of at least 50 percent can be measured. The ALAD enzyme is associated with the formation of hemoglobin in blood and in chemical detoxification processes in the liver. This biological response is a measure of the rate of ALAD activity. This biological response may be used to determine injury to bird and mammal species that have been exposed to lead.

(E) *Reduced fish reproduction.* Injury has occurred when a statistically significant difference in reproduction success between the control organisms and the test organisms can be measured based on the use of published standardized laboratory toxicity testing methodologies. This biological response may be used when the oil or hazardous substance is suspected to have caused a reduction in the reproductive success of fish species. Laboratory partial-chronic and laboratory chronic toxicity tests may be used. The oil or hazardous substance used in the test must be the exact substance or a substance that is reasonably comparable to that suspected to have caused reduced reproductive success in the natural population of fish.

(vi) *Category of injury—physical deformation.* Four biological responses for determining when physical deformations are a result of exposure to the discharge of oil or release of a hazardous substance have met the injury acceptance criteria.

(A) *Overt external malformations.* Injury has occurred when a statistically significant difference can be measured in the frequency of overt external malformation, such as small or missing eyes, when comparing samples from populations of wildlife species from the assessment area and a control area. This biological response may be used as a demonstration of injury when such physical deformations are observed in wildlife species exposed to oil or hazardous substances.

(B) *Skeletal deformities.* Injury has occurred when a statistically significant difference can be measured in the frequency of skeletal deformities, such as defects in growth of bones, when comparing samples from populations of wildlife species from the assessment area and a control area. This biological response may be used as a demonstration of injury when such physical deformations are observed in wildlife species exposed to oil or hazardous substances.

(C) *Internal whole organ and soft tissue malformation.* Injury has occurred when a statistically significant difference can be measured in the frequency of malformations to brain, heart, liver, kidney, and other organs, as well as soft tissues of the gastrointestinal tract and vascular system, when comparing samples from populations of wildlife species in the assessment area and a control area. This biological response may be used as a demonstration of injury when such physical deformations are observed in wildlife species exposed to oil or hazardous substances.

(D) *Histopathological lesions.* Injury has occurred when a statistically significant difference can be measured in the frequency of tissue or cellular lesions when comparing samples from populations of wildlife species from the assessment area and a control area. This biological response may be used as a demonstration of injury when such physical deformations are observed in wildlife species exposed to oil or hazardous substances.

§ 11.63 Injury determination phase—pathway determination.

(a) *General.* (1) To determine the exposure pathways of the oil or hazardous substance, the following shall be considered:

(i) The chemical and physical characteristics of the discharged oil or released hazardous substance when transported by natural processes or while present in natural media;

(ii) The rate or mechanism of transport by natural processes of the discharged oil or released hazardous substance; and

(iii) Combinations of pathways that, when viewed together, may transport the discharged oil or released hazardous substance to the resource.

(2) The pathway may be determined by either demonstrating the presence of the oil or hazardous substance in sufficient concentrations in the pathway resource or by using a model that

demonstrates that the conditions existed in the route and in the oil or hazardous substance such that the route served as the pathway.

(3) To the extent that the information needed to make this determination is not available, tests shall be conducted and necessary data shall be collected to meet the requirements of this section. Methods that may be used to conduct these additional tests and collect new information are described in §11.64 of this part.

(b) *Surface water pathway.* (1) When the surface water resource is suspected as the pathway or a component of the pathway, the authorized official shall determine, using guidance provided in this paragraph, whether the surface water resource, either solely or in combination with other media, served as the exposure pathway for injury to the resource.

(2)(i) Using available information and such additional tests as necessary, it should be determined whether the surface water resource downstream or downcurrent of the source of discharge or release has been exposed to the oil or hazardous substance.

(ii) When the source of discharge or release is on an open water body, such as a marsh, pond, lake, reservoir, bay, estuary, gulf, or sound, it should be determined, using available information and such additional tests as necessary, whether the surface water resource in the vicinity of the source of discharge or release has been exposed to the oil or hazardous substance.

(3)(i) If a surface water resource is or likely has been exposed, the areal extent of the exposed surface water resource should be estimated, including delineation of:

(A) Channels and reaches:

(B) Seasonal boundaries of open water bodies; and

(C) Depth of exposed bed, bank, or shoreline sediments.

(ii) As appropriate to the exposed resource, the following should be determined:

(A) Hydraulic parameters and streamflow characteristics of channels and reaches;

(B) Bed sediment and suspended sediment characteristics, including grain size, grain mineralogy, and chemistry of grain surfaces;

(C) Volume, inflow-outflow rates, degree of stratification, bathymetry, and bottom sediment characteristics of surface water bodies;

(D) Suspended sediment concentrations and loads and bed forms and loads of streams and tidally affected waters; and

(E) Tidal flux, current direction, and current rate in coastal and marine waters.

(4)(i) Using available information and data from additional tests as necessary, the mobility of the oil or hazardous substance in the exposed surface water resource should be estimated. This estimate should consider such physical and chemical characteristics of the oil or hazardous substance as aqueous solubility, aqueous miscibility, density, volatility, potential for chemical degradation, chemical precipitation, biological degradation, biological uptake, and adsorption.

(ii) Previous studies of the characteristics discussed in paragraph (b)(4)(i) of this section should be relied upon if hydraulic, physical, and chemical conditions in the exposed surface water resource are similar to experimental conditions of the previous studies. In the absence of this information, those field and laboratory studies necessary to estimate the mobility of the oil or hazardous substance in surface water flow may be performed.

(5)(i) The rate of transport of the oil or hazardous substance in surface water should be estimated using available information and with consideration of the hydraulic properties of the exposed resource and the physical and chemical characteristics of the oil or hazardous substance.

(ii) Transport rates may be estimated using:

(A) The results of previous time-of-travel and dispersion studies made in the exposed surface water resource before the discharge or release;

(B) The results of previous studies, conducted with the same or similar chemical substances to those discharged or released under experimental conditions similar to the hydraulic, chemical, and biological conditions in the exposed surface water resource;

(C) The results of field measurements of time-of-travel and dispersion made in the exposed or comparable surface water resource, using natural or artificial substances with transport characteristics that reasonably approximate those of the oil or hazardous substance; and

(D) The results of simulation studies using the results of appropriate time-of-travel and dispersion studies in the exposed or comparable surface water resource.

(c) *Ground water pathway.* (1) When ground water resources are suspected as the pathway or a component of the pathway, the authorized official shall determine, using guidance provided in this paragraph, whether ground water resources, either solely or in combination with other media, served as the exposure pathway for injury to the resource.

(2) Using available information and such additional tests as necessary, it should be determined whether the unsaturated zone, the ground water, or the geologic materials beneath or downgradient of the source of discharge or release have been exposed to the oil or hazardous substance.

(3) If a ground water resource is or likely has been exposed, available information and such additional tests should be used as necessary to determine the characteristics of the unsaturated zone, as well as any aquifers and confining units containing the exposed ground water, in the vicinity of the source of discharge or release. The characteristics of concern include:

(i) Local geographical extent of aquifers and confining units;

(ii) Seasonal depth to saturated zone beneath the site;

(iii) Direction of ground water flow in aquifers;

(iv) Local variation in direction of ground water flow resulting from seasonal or pumpage effects;

(v) Elevation of top and bottom of aquifer and confining units;

(vi) Lithology, mineralogy, and porosity of rocks or sediments comprising the unsaturated zone, aquifers, and confining units;

(vii) Transmissivity and hydraulic conductivity of aquifers and confining units; and

(viii) Nature and amount of hydraulic connection between ground water and local surface water resources.

(4)(i) Using available information and such additional tests as necessary, the mobility of the oil or hazardous substance within the unsaturated zone and in the exposed ground water resources should be estimated. This estimate should consider local recharge rates and such physical and chemical characteristics of the oil or hazardous substance as aqueous solubility, aqueous miscibility, density, volatility, potential for chemical degradation, chemical precipitation, biological degradation, biological uptake, and adsorption onto solid phases in the unsaturated zone, aquifers, and confining units.

(ii) Previous studies of the characteristics discussed in paragraph (c)(4)(i) of this section should be relied upon if geohydrologic, physical, and chemical conditions in the exposed ground water resource are similar to experimental conditions of the previous studies. In the absence of this information, field and laboratory studies may be performed as necessary to estimate the mobility of the oil or hazardous substance within the unsaturated zone and in ground water flows.

(5)(i) The rate of transport of the oil or hazardous substance in ground water should be estimated using available information and with consideration of the site hydrology, geohydrologic properties of the exposed resource, and the physical and chemical characteristics of the oil or hazardous substance.

(ii) Transport rates may be estimated using:

(A) Results of previous studies conducted with the same or similar chemical substance, under experimental geohydrological, physical, and chemical conditions similar to the ground water resource exposed to the oil or hazardous substance;

(B) Results of field measurements that allow computation of arrival times of the discharged or released substance at downgradient wells, so that an empirical transport rate may be derived; or

(C) Results of simulation studies, including analog or numerical modeling of the ground water system.

(d) *Air pathway.* (1) When air resources are suspected as the pathway or a component of the pathway, the authorized official shall determine, using guidance provided in this paragraph, whether the air resources either solely or in combination with other media, served as the exposure pathway for injury to the resource.

(2) Using available information, air modeling, and additional field sampling and analysis, it should be determined whether air resources have been exposed to the discharge of oil or release of a hazardous substance.

(3)(i) If an air resource is or has likely been exposed, available information and such additional tests as necessary should be used to estimate the areal extent of exposure and the duration and frequency of exposure of such areas to emissions from the discharge of oil or release of a hazardous substance.

(ii) The areal extent of exposure is defined as the geographical surface area or space where emissions from the source of discharge or release are found or otherwise determined to be present for such duration and frequency as to potentially result in injury to resources present within the area or space.

(4) Previous studies of the characteristics discussed in paragraph (d)(3)(i) of this section should be relied upon if the conditions in the exposed air resource are similar to experimental conditions of the previous studies. In the absence of this information, air sampling and analysis methods identified in §11.64(d) of this part, air modeling methods, or a combination of these methods may be used in identifying the air exposure pathway and in estimating the areal extent of exposure and duration and frequency of exposure.

(5) For estimating the areal extent, duration, and frequency of exposure from the discharge or release, the following factors shall be considered as may be appropriate for each emissions event:

(i) The manner and nature in which the discharge or release occurs, including the duration of the emissions, amount of the discharge or release, and emergency or other time critical factors;

(ii) The configuration of the emitting source, including sources such as ponds, lagoons, pools, puddles, land and water surface spills, and venting from containers and vessels;

(iii) Physical and chemical properties of substances discharged or released, including volatility, toxicity, solubility, and physical state;

(iv) The deposition from the air and re-emission to the air of gaseous and particulate emissions that provide periodic transport of the emissions; and

(v) Air transport and dispersion factors, including wind speed and direction, and atmospheric stability and temperature.

(e) *Geologic pathway.* (1) When geologic resources are suspected as the pathway or a component of the pathway, the authorized official shall determine, using guidance provided in this paragraph, whether geologic resources, either solely or in combination with other media, served as the exposure pathway for injury to the resource.

(2)(i) Using available information and the methods listed in §11.64(e) of this part, it should be determined whether any element of the geologic resource has been exposed to the oil or hazardous substance. If a geologic resource is or has likely been exposed, the areal extent of the exposed geologic resource, including the lateral and vertical extent of the dispersion, should be estimated.

(ii) To determine whether the unsaturated zone served as a pathway, the guidance provided in paragraph (c) of this section should be followed.

(f) *Biological pathway.* (1) When biological resources are suspected as the pathway or a component of the pathway, the authorized official shall determine, using the guidance provided in this paragraph, whether biological resources, either solely or in combination with other media, served as the exposure pathway for injury to the resource.

(2) Biological pathways that resulted from either direct or indirect exposure to the oil or hazardous substance, or from exposure to products of chemical or biological reactions initiated by the discharge or release shall be identified. Direct exposure can result from direct

physical contact with the discharged oil or released hazardous substance. Indirect exposure can result from food chain processes.

(3) If the oil or hazardous substance adhered to, bound to, or otherwise covered surface tissue, or was ingested, or inhaled but not assimilated, the area of dispersion may be determined based upon chemical analysis of the appropriate tissues or organs (such as leaves, lungs, stomach, intestine, or their contents) that were directly exposed to the oil or hazardous substance.

(4) If the oil or hazardous substance was assimilated, the areal dispersion may be determined based upon one or more of the following alternative procedures:

(i) If direct exposure to the biological resource has occurred, chemical analysis of the organisms that have been exposed may be performed.

(ii) If indirect exposure to the biological resource has occurred, either chemical analysis of free-ranging biological resources using one or more indicator species as appropriate, or laboratory analysis of one or more in situ placed indicator species as appropriate may be performed.

(A) *Indicator species*, as used in this section, means a species of organism selected consistent with the following factors to represent a trophic level of a food chain:

(*1*) General availability of resident organisms in the assessment area;

(*2*) Potential for exposure to the oil or hazardous substance through ingestion, assimilation, or inhalation;

(*3*) Occurrence of the substance in a chemical form that can be assimilated by the organism;

(*4*) Capacity of the organism to assimilate, bioconcentrate, bioaccumulate, and/or biomagnify the substance;

(*5*) Capacity of the organism to metabolize the substance to a form that cannot be detected through available chemical analytical procedures; and

(*6*) Extent to which the organism is representative of the food chain of concern.

(B) Collection of the indicator species should be limited to the number necessary to define the areal dispersion and to provide sufficient sample volume for chemical analysis.

(C) When in situ procedures are used, indicator species that behave comparably to organisms existing under free-ranging conditions shall be collected. The indicator species used in this procedure shall be obtained either from a control area selected consistent with provisions of § 11.72 of this part or obtained from a suitable supply of wild-strain organisms reared in a laboratory setting. Appropriate chemical analysis shall be performed on a representative subsample of the indicator species before in situ placement.

(iii) In situ placement procedures shall be used where the collection of samples would be inconsistent with the provisions of § 11.17(b) of this part.

(5) Sampling sites and the number of replicate samples to be collected at the sampling sites shall be consistent with the quality assurance provisions of the Assessment Plan.

(6) Chemical analysis of biological resource samples collected for the purpose of this section shall be conducted in accordance with the quality assurance provisions of the Assessment Plan.

§ 11.64 Injury determination phase—testing and sampling methods.

(a) *General*. (1) The guidance provided in this section shall be followed for selecting methodologies for the Injury Determination phase.

(2) Before selecting methodologies, the objectives to be achieved by testing and sampling shall be defined. These objectives shall be listed in the Assessment Plan. In developing these objectives, the availability of information from response actions relating to the discharge or release, the resource exposed, the characteristics of the oil or hazardous substance, potential physical, chemical, or biological reactions initiated by the discharge or release, the potential injury, the pathway of exposure, and the potential for injury resulting from that pathway should be considered.

(3) When selecting testing and sampling methods, only those methodologies shall be selected:

(i) For which performance under conditions similar to those anticipated at the assessment area has been demonstrated;