§ 20.134 Nontoxic shot.

(a) *Approval.* (1) The information collection requirements contained in §20.134 have been approved by the Office of Management and Budget under 44 U.S.C. 3501 *et seq.* and assigned clearance number 1018–0067. The information is being collected to provide a basis for which the Director, Fish and Wildlife Service, can conduct a methodical and objective review to approve/disapprove nontoxic shot status sought by an applicant. The information will be used for toxicity assessment of candidate shot submitted for approval by applicant. Response is required to obtain a benefit.

(2) The Director, U.S. Fish and Wildlife Service, shall determine that a specific type of shot material is acceptable for the purposes of 50 CFR 20.21(j), if after a review of applications and supporting data submitted in accordance with this section, together with all other relevant evidence, including public comment, it is concluded that the spent shot material does not impose a significant danger to migratory birds and other wildlife or their habitats.

(b) *Application and review—Tiered strategy for approval of nontoxic shot and shot coating.* (1) All applications for approval under this section must be submitted with supporting documentation to the Director in accordance with the following procedures and must include at a minimum the supporting materials and information covered by Tier 1 in the tiered approval system as follows:

(2) *Tier 1.* (i)(A) Applicant provides statements of use, chemical characterization, production variability, volume of use of candidate material and shot sample as listed in paragraphs (b)(2)(i)(A)(*1*) through (*5*), (b)(2)(i)(B)(*1*) through (*5*), and (b)(2)(i)(C)(*1*) through (*3*) of this section. The candidate shot or shot coating may be chemically analyzed by the Service or an independent laboratory to compare the results with the applicant's descriptions of shot composition and composition variability. Rejection of the application will occur if it is incomplete or if the composition of the candidate material, upon analysis, varies significantly from that described by the applicant.

(*1*) Statement of proposed use, i.e., purpose and types.

(*2*) Description of the chemical composition of the intact material.

(*i*) Chemical names, Chemical Abstracts Service numbers (if available), and structures.

(*ii*) Chemical characterization for organics and organometallics for coating and core [e.g., empirical formula, melting point, molecular weight, solubility, specific gravity, partition

coefficients, hydrolysis half-life, leaching rate (in water and soil), degradation half-life, vapor pressure, stability and other relevant characteristics].

(*iii*) Composition and weight of shot material.

(*iv*) Thickness, quantity (e.g., mg/shot), and chemical composition of shot coating.

(*3*) Statement of the expected variability of shot during production.

(*4*) Estimate of yearly volume of candidate shot and/or coated shot expected for use in hunting migratory birds in the U.S.

(*5*) Five pounds of the candidate shot and/or coated shot, as applicable, in size equivalent to United States standard size No. 4 (0.13 inches in diameter).

(B) Applicant provides information on the toxicological effects of the shot or shot coating as follows:

(*1*) A summary of the acute and chronic mammalian toxicity data of the shot or shot coating ranking its toxicity (e.g., LD50<5 mg/kg = super toxic, 5–50 mg/kg = extremely toxic, 50–500 mg/kg = very toxic, 500–5,000 mg/kg = moderately toxic, 5,000–15,000 = slightly toxic, >15,000 mg/kg = practically nontoxic) with citations.

(*2*) A summary of known acute, chronic, and reproductive toxicological data of the chemicals comprising the shot or shot coating with respect to birds, particularly waterfowl (include LD50 or LC50 data, and sublethal effects) with citations.

(*3*) A narrative description, with citations to relevant data, predicting the toxic effect in waterfowl of complete erosion and absorption of one shot or coated shot in a 24-hour period. Define the nature of toxic effect (e.g., mortality, impaired reproduction, substantial weight loss, disorientation and other relevant associated clinical observations).

(*4*) A statement, with supporting rationale and citations to relevant data, that there is or is not any reasonable basis for concern for shot or coated shot ingestion by fish, amphibians, reptiles or mammals. If there is some recognized impact on fish, amphibians, reptiles, or mammals, the Service may require additional study.

(*5*) Summarize the toxicity data of chemicals comprising the shot or shot coating to aquatic and terrestrial invertebrates, fish, amphibians, reptiles, and mammals.

(C) Applicant provides information on the environmental fate and transport, if any, of the shot or shot coating as follows:

(*1*) A statement of the alteration of the shot or shot coating, chemically or physically, upon firing. The statement must describe any alterations.

(*2*) An estimate of the environmental half-life of the organic or organometallic component of the shot or shot coating, and a description of the chemical form of the breakdown products.

(*3*) Information on the Estimated Environmental Concentration (EEC) assuming 69,000 shot per hectare (Bellrose 1959; Pain 1990) for:

(*i*) A terrestrial ecosystem, assuming complete dissolution of material in 5 cm of soil. What would be the EEC and would that EEC exceed existing clean soil standards? (Environmental Protection Agency [EPA] standards for the Use of Disposal of Sewage Sludge; 40 CFR Part 503). How does the estimated EEC relate to the toxicity threshold for plants, invertebrates, fish and wildlife?

(*ii*) An aquatic ecosystem, assuming complete dissolution of the shot or shot coating in 1 cubic foot of water. What is the estimated EEC, and how does it compare to the EPA Water Quality Criteria and toxicity thresholds in plants, invertebrates, fish and wildlife?

(D) *Service evaluation of an application.* (*1*) In reviewing the submission, the Service will use an exceedence of 1 LD50/square foot as the level of concern (U.S.E.P.A. 1992) as a criteria in the risk assessment.

(*2*) In cooperation with the applicant, the Service will conduct a risk assessment using the Quotient Method (Environmental Protection Agency 1986): Risk = EEC/Toxicological Level of Concern Compare EEC in ppm to an effect level (e.g., LD50 in ppm. If $Q < 0.1$ = No Adverse Effects; If $0.1 \leq Q \leq 10.0$ = Possible Adverse Effects; If $Q > 10.0$ = Probable Adverse Effects.

(*3*) Upon receipt of the Tier 1 application, the Director will review it to determine if the submission is complete. If complete, the applicant is notified within 30 days of receipt that a thorough review of the application will commence. A *Notice of Application* will appear in the Federal Register announcing the initiation of review of a Tier 1 application. Complete review of a Tier 1 application will occur within 60 days of the date the *Notice of Application* is published in the Federal Register.

(E) If, after review of the Tier 1 data, the Service does not conclude that the shot or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, the applicant is advised to proceed with the additional testing described for Tier 2, Tier 3, or both. A *Notice of Review* will inform the public that Tier 1 test results are inconclusive, and Tier 2, Tier 3, or both testing are required before further consideration.

(F) If review of the Tier 1 data results in a preliminary determination that the candidate material does not impose a significant danger to migratory birds, other wildlife, and their habitats, the Director will publish in the Federal Register a proposed rule stating the Service's intention to approve this shot or shot coating based on the toxicological report and toxicity studies. The rulemaking will include a description of the chemical composition of the candidate shot or shot coating, and a synopsis of findings under the standards required for Tier 1. If, at the end of the comment period, the Service finds no technical or scientific basis upon which to alter its conclusion, the candidate material will be approved by the publication of a final rule in the

Federal Register. If, after receiving public comment, the Service determines that all available information does not establish that the shot and/or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, Tier 2, Tier 3, or both testing will be required and a *Notice of Review* will appear in the Federal Register. If only one of these two Tier tests are required, the Service will explain in the notice why the other is not required. If the applicant chooses not to proceed, the determination denying approval will appear in the Federal Register.

(ii) [Reserved]

(3) *Tier 2.* (i) If Tier 2 testing is required, the applicant must submit a plan that addresses paragraph (b)(3)(ii) requirements. The Director will review the Tier 2 testing plan submitted by the applicant within 30 days of receipt. The Director may decline to approve the plan, or any part of it, if deficient in any manner with regard to timing, format or content. The Director shall apprise the applicant regarding what parts, if any, of the submitted testing procedures to disregard and any modifications to incorporate into the Tier 2 testing plan in order to gain plan approval. All testing procedures will be in compliance with the Good Laboratory Practices Standards (40 CFR part 160) except where they conflict with the regulations in this section or with a provision of an approved plan. The Director, or authorized representative, may elect to inspect the applicant's laboratory facilities and may decline to approve the plan and further consideration of the candidate shot if the facility does not meet the Good Laboratory Practices Standards. After the plan is accepted, Tier 2 testing will commence. Required analyses and reports, in accordance with the regulations in this section, must be sent to the Director. The applicant will ensure that copies of all the raw data and statistical analyses accompany the laboratory reports and final comprehensive report of this test.

(ii) Evaluation of the candidate shot or shot coating will first be in a standardized test under *in vitro* conditions (see paragraph be in a standardized test under *in vitro* conditions (see paragraph (b)(3)(ii)(A)) that will assess its erosion and any release of components into a liquid medium in an environment simulating *in vivo* conditions of a waterfowl gizzard. Erosion characteristics are to be compared with those of lead shot and steel shot of comparable size. Following the erosion rate testing, the applicant must conduct a 30-day acute toxicity test in mallards, and a test to determine the candidate shot and/or shot coating effects on selected invertebrates and fish and include the results in the report for the Director.

(A) *In vitro erosion rate test.* Conduct a standardized *in vitro* test to determine erosion rate of the candidate shot or shot coating using the guidelines in Kimball and Munir (1971), unless otherwise provided by the Service.

(*1*) *Typical test materials:* Atomic absorption spectrophotometer; Drilled aluminum block to support test tubes; Thermostatically controlled stirring hot plate; Small Teflon®-coated magnets; Hydrochloric acid (pH 2.0) and pepsin; Capped test tubes; and Lead, steel and candidate shot/coated shot.

(*2*) *Typical test procedures.* Add hydrochloric acid and pepsin to each capped test tube at a volume and concentration that will erode a single No. 4 lead shot at a rate of 5 mg/day. Place

three test tubes, each containing either lead shot, steel shot or candidate shot and/or coated shot, in an aluminum block on the stirring hot plate. Add a Teflon® coated magnet to each test tube and set the hot plate at 42 degrees centigrade and 500 revolutions per minute. Determine the erosion of shot or coated shot daily for 14 consecutive days by weighing the shot and analyzing the digestion solution with an atomic absorption spectrophotometer. Replicate the 14-day procedure five times.

(*3*) *Typical test analyses.* Compare erosion rates of the three types of shot by appropriate analysis of variance and regression procedures. The statistical analysis will determine whether the rate of erosion of the shot and/or shot coating is significantly greater or less than that of lead and steel. This determination is important to any subsequent toxicity testing.

(B) *Acute toxicity test—Tier 2 (Short-term, 30-day acute toxicity test using a commercially available duck food.).* Over a 30-day period, conduct a short-term acute toxicity test that complies with the guidelines described as follows or as otherwise provided by the Service:

(*1*) *Typical test materials:* 30 male and 30 female hand-reared mallards approximately 6 to 8 months old (mallards must have plumage and body conformation that resemble wild mallards); 60 elevated outdoor pens equipped with feeders and waterers; Laboratory equipped to perform fluoroscopy, required blood and tissue assays, and necropsies; Commercial duck maintenance mash; and Lead, steel and candidate shot.

(*2*) *Typical test procedures.* House mallards individually in pens and give *ad libitum* access to food and water. After 3 weeks, randomly assign to 3 groups (10 males and 10 females/group), dose with eight pellets of either No. 4 lead shot (positive control), steel shot (negative control), or the candidate shot or coated shot. Fluoroscope birds at 1 week after dosage to check for shot retention. Observe birds daily for signs of intoxication and mortality over a 30-day period. Determine body weight at the time of dosing, and at days 15 and 30 of the test. On days 15 and 30, collect blood by venipuncture, determine hematocrit, hemoglobin concentration and other specified blood chemistries. Sacrifice all survivors on day 30. Remove the liver and other appropriate organs from the sacrificed birds and from birds that died prior to sacrifice on day 30 for histopathological analysis. Analyze the organs for lead and compounds contained in the candidate shot or coated shot. Necropsy all birds to determine any pathological conditions.

(*3*) *Typical test analyses.* Analyze mortality among the specified groups with appropriate chi-square statistical procedures. Analyze physiological data and tissue contaminant data by analysis of variance or other appropriate statistical procedures to include the factors of shot type and sex. Compare sacrificed birds and birds that died prior to sacrifice whenever sample sizes are adequate for meaningful comparison.

(C) *Daphnid and fish early-life toxicity tests.* Determine the toxicity of the compounds that comprise the shot or shot coating (at conditions maximizing solubility without adversely affecting controls) to selected invertebrates and fish. These methods are subject to the environmental effects test regulations developed under the authority of the Toxic Substances Control Act (15 U.S.C. 2601 *et seq.*), as follows:

(*1*) The first test, the *Daphnid Acute Toxicity Test* (conducted in accordance with 40 CFR 797.1300), is a guideline for use in developing data on the acute toxicity of chemical substances. This guideline prescribes an acute toxicity test in which Daphnid exposure to a chemical in static and flow-through systems, with the agencies assessing the hazard the compound(s) may present to an aquatic environment.

(*2*) The second test is the *Daphnid Chronic Toxicity Test* (conducted in accordance with 40 CFR 797.1330). This gathers data on the chronic toxicity of chemical substances in which Daphnids (*Daphnia* spp.) are exposed to a chemical in a renewal or flow-through system. The data from this test are again used to assess the hazard that the compound(s) may present to an aquatic environment.

(*3*) A third test, *Fish Early Life Stage Toxicity Test* (conducted in accordance with 40 CFR Section 797.1600), assesses the adverse effects of chemical substances to fish in the early stages of their growth and development. Data from this test are used to determine the hazard the compound(s) may present to an aquatic environment.

(iii) After the Tier 2 testing, the applicant will report the results to the Director. If, after review of the Tier 2 data, the Service determines that the information does not establish that the shot or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, the applicant is advised to proceed with the additional testing in Tier 3. A *Notice of Review* advises the public that, in conjunction with Tier 1 data, Tier 2 test results are inconclusive and Tier 3 testing is required for continued consideration.

(iv) If review of the Tier 2 test data results in a preliminary determination that the candidate shot or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, the Director will publish in the Federal Register a proposed rule stating the Service's intention to approve this shot and/or coating and why Tier 3 testing is unnecessary. The rulemaking will include a description of chemical composition of the shot or shot coating, and a synopsis of findings under the standards required at Tier 2. If, at the end of the comment period, the Service finds no technical or scientific basis upon which to deny approval, the candidate shot or shot coating approval is published as a final rule in the Federal Register. If, as a result of the comment period, the Service determines that the information does not establish that the shot and/or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, Tier 3 testing will be required and a *Notice of Review* published in the Federal Register. If the applicant chooses not to proceed, the determination denying approval of the candidate shot or shot coating will appear in the Federal Register.

(4) *Tier 3.* (i) If the Director determines that the Tier 1 or Tier 2 information is inconclusive, the Director will notify the applicant to submit a Tier 3 testing plan for conducting further testing as outlined in paragraphs (b)(4)(i) (A) and (B) of this section. Review, by the Director, of the Tier 3 testing plan submitted by the applicant will occur within 30 days of receipt. The Director may decline to approve the plan, or any part of it, if deficient in any manner with regard to timing, format or content. The Director shall apprise the applicant regarding what parts, if any, of the submitted testing procedure to disregard and any modifications to incorporate into the Tier 3 plan in order to gain plan approval. All testing procedures should be in compliance with the Good

Laboratory Practices Standards (40 CFR part 160), except where they conflict with the regulations in this section or with a provision of an approved plan. The Director, or authorized representative, may elect to inspect the applicant's laboratory facilities and may decline to approve the plan and further consideration of the candidate shot and/or shot coating if the facility is not in compliance with the Good Laboratory Practices Standards. After acceptance of the plan, Tier 3 testing will commence. Required analyses and reports must be sent to the Director. The applicant will ensure that copies of all the raw data and statistical analyses accompany the laboratory reports and final comprehensive report of this test.

(A) *Chronic toxicity test—Tier 3 (Long-term toxicity test under depressed temperature conditions using a nutritionally-deficient diet).* Conduct a chronic exposure test under adverse conditions that complies with the general guidelines described as follows unless otherwise provided by the Service:

(*1*) *Typical test materials:* 36 male and 36 female hand-reared mallards approximately 6 to 8 months old (Mallards must have plumage and body conformation that resembles wild mallards); 72 elevated outdoor pens equipped with feeders and waterers; Laboratory equipped to perform fluoroscopy, required blood and tissue assays, and necropsies; Whole kernel corn; and Lead, steel, and candidate shot or coated shot.

(*2*) *Typical test procedures.* (*i*) Conduct this test at a location where the mean monthly low temperature during December through March is between 20 and 40 degrees Fahrenheit (−6.6 and 4.4 degrees centigrade, respectively). Assign individual mallards to elevated outdoor pens during the first week of December and acclimate to an *ad libitum* diet of whole kernel corn for 2 weeks. Randomly assign birds to 5 groups (lead group of 4 males and 4 females, 4 other groups of 8 males and 8 females/group). Dose the lead group (positive control) with one size No. 4 pellet of lead shot. Dose one group (8 males and 8 females) with eight size No. 4 pellets of steel shot (negative control) and dose the 3 other groups (8 males and 8 females/group) with one, four and eight size No. 4 pellets of candidate shot or coated shot.

(*ii*) Weigh and fluoroscope birds weekly. Weigh all recovered shot to measure erosion. Determine blood parameters given in the 30-day acute toxicity test. Provide body weight and blood parameter measurements on samples drawn at 24 hours after dosage and at the end of days 30 and 60. At the end of 60 days, sacrifice all survivors. Remove the liver and other appropriate organs from sacrificed birds and birds dying prior to sacrifice on day 60 for histopathological analysis. Analyze organs for lead and other metals potentially contained in the candidate shot or shot coating. Necropsy all birds that died prior to sacrifice to determine pathological conditions associated with death.

(*3*) *Typical test analyses.* Analyze mortality among the specified groups with appropriate chi-square statistical procedures. Any effects on the previously mentioned physiological parameters caused by the shot or shot coating must be significantly less than those caused by lead shot and must not be significantly greater than those caused by steel shot. Analyze physiological data and tissue contaminant data by analysis of variance or appropriate statistical procedures to include the factors of shot type, dose and sex. Compare sacrificed birds and birds that died prior to sacrifice whenever sample sizes are adequate for a meaningful comparison.

(B) *Chronic dosage study—Tier 3 (Moderately long-term study that includes reproductive assessment).* Conduct chronic exposure reproduction trial with the general guidelines described as follows unless otherwise provided by the Service:

(*1*) *Typical test materials:* 44 male and 44 female hand-reared first year mallards (Mallards must have plumage and body conformation that resemble wild mallards); Pens suitable for quarantine and acclimation and for reasonably holding 5–10 ducks each; 44 elevated, pens equipped with feeders, waterers and nest boxes; Laboratory equipped to perform fluoroscopy, required blood and tissue assays, and necropsies; Whole kernel corn, and commercial duck maintenance and breeder mash; and Lead, steel and candidate shot or coated shot.

(*2*) *Typical test procedures.* (*i*) Randomly assign mallards to 3 groups (Lead group = 4 males and 4 females; steel group = 20 males and 20 females; candidate shot/coated shot group = 20 males and 20 females) in December and hold in same-sex groups until mid-January (dates apply to outdoor test facility only and will reflect where in the U.S. tests are conducted). Tests conducted in the southern U.S. will need to be completed in low temperature units. After a 3-week acclimation period with ducks receiving commercial maintenance mash, provide birds with an *ad libitum* diet of corn for 60 days and then pair birds (one pair/pen) and provide commercial breeder mash. Dosing of the 3 groups with one pellet of No. 4 lead shot (positive control); eight pellets of No. 4 steel shot (negative control); and eight pellets of No. 4 candidate shot or coated shot will occur after the acclimation period (day 0) and redosed after 30, 60, and 90 days. Few, if any, of the lead-dosed birds (positive control) should survive and reproduce.

(*ii*) Fluoroscope birds 1 week after dosage to check for shot retention. Weigh males and females the day of initial dosing (day 0), at each subsequent dosing, and at death. Measure blood parameters identified in the 30-Day Acute Toxicity Test in this test using samples drawn at time of weighing. Note the date of first egg and the mean number of days per egg laid. Conclude laying after 21 normal, uncracked eggs are laid or after 150 days. Sacrifice adults after completion of laying period. Remove the liver and other appropriate organs from sacrificed birds and from other birds that died prior to sacrifice for histopathological analysis. Analyze organs and the 11th egg for compounds contained in the shot or shot coating. Necropsy all birds to determine any pathological conditions. Check nests daily to collect eggs. Discard any eggs laid before pairing. Artificially incubate eggs and calculate the percent shell thickness, percent eggs cracked, percent fertility (as determined by candling), and percent hatch of fertile eggs for each female. Provide ducklings with starter mash after hatching. Sacrifice all ducklings at 14 days of age. Measure survival to day 14 and weight of the ducklings at hatching and sacrifice. Measure blood parameters identified in the 30-Day Acute Toxicity Test using samples drawn at sacrificing.

(*3*) *Typical test analyses.* (*i*) Any mortality, reproductive inhibition or effects on the physiological parameters in paragraph (b)(4) by the shot or shot coating must not be significantly greater than those caused by steel shot. Percentage data is subject to an arcsine, square root transformation prior to statistical analyses. Physiological and reproductive data is analyzed by one-tailed *t*-tests (α=0.05), or other appropriate statistical procedures by the applicant.

(*ii*) After conclusion of Tier 3 testing, the applicant must report the results to the Director. If after review of the Tier 3 data (completion 60 days after receipt of material) the Service determines that all of the information gathered and submitted in accordance with Tiers 1, 2, and 3, as applicable, does not establish that the shot or shot coating does not impose a significant danger to migratory birds, other wildlife, and their habitats, the applicant will have the option of repeating the tests that the Director deems are inconclusive. If the applicant chooses not to repeat the tests, approval of the candidate shot or shot coating is denied. A *Notice of Review* will inform the public that Tier 3 results are inconclusive, the applicant's decision not to repeat Tier 3 testing, and the Service's subsequent denial of the shot or shot coating.

(*iii*) If review of either the initial or repeated Tier 3 test data results in a preliminary determination that the shot or shot coating does not impose a significant danger to migratory birds, other wildlife and their habitats, the Director will publish in the Federal Register a proposed rule stating the Service's intention to approve this shot or shot coating and providing the public with the opportunity to comment. The rulemaking will include a description of the chemical composition of the shot or shot coating and a synopsis of findings under the standards required by Tier 3. If at the end of the comment period, the Service concludes that the shot or shot coating does not impose a significant danger to migratory birds, other wildlife, or their habitats, the shot or shot coating will be approved as nontoxic with publication of a final rule in the Federal Register.

(5) *Residual lead levels.* The Service's maximum environmentally acceptable level of lead in shot is trace amounts or <1 percent. Any shot manufactured with lead levels equal to or exceeding 1 percent are considered toxic and, therefore, illegal.

(6) *Field detection device.* Before approval of any shot for use in migratory game bird hunting, a noninvasive field testing device must be available for enforcement officers to determine the shot material in a given shell in the field.

(Information collection requirements approved by the Office of Management and Budget under control no. 1018–0067)

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