

(iv) Sample temperature must be maintained between 4 at 15 °C. during processing. Use separate sets of sterile homogenization and processing equipment to process fluids or tissues from each fish lot sampled. Processing equipment need not be sterilized between samples within a single lot.

(v) Homogenized tissue samples may be diluted 1:10 with buffered cell culture medium (pH 7.4–7.8) containing antibiotics and antifungal agents not exceeding the concentrations described in paragraph (e)(2)(iii) of this section. Centrifuge tissue suspensions and ovarian fluid samples 4 °C. at 2,500 × gravity (g) (relative centrifugal force) for 15 minutes. Resulting supernatant solutions can be stored overnight at 4 °C.

(vi) At the time of inoculation onto cell cultures, total dilution of processed tissue samples must not exceed 1:100 ((volume to volume) (v/v)); total dilution of ovarian fluid samples must not exceed 1:20 (v/v). In samples inoculated onto cell cultures, the final antibiotic concentration shall not exceed 100 µg/ml of Gentamicin, 100 IU/ml of penicillin, or 100 µg/ml of streptomycin and antifungal agent concentrations should not exceed 25 IU/ml of mycostatin (Nystatin) or 2.5 µg/ml of amphotericin B (Fungizone).

(3) *Cell culture procedures.* (i) Both epithelioma papulosum cyprini (EPC) and chinook salmon embryo (CHSE-214) cell lines must be maintained and used in all virus assays. Susceptible, normal appearing, and rapidly dividing cell cultures shall be selected. Penicillin (100 IU/ml), streptomycin (100 µg/ml), and antifungal agents, such as mycostatin/Nystatin (25 IU/ml) or amphotericin B/Fungizone (2.5 µg/ml), are permitted in media used for cell culture and virus assay work.

(ii) Cell cultures shall be seeded and grown, at optimum temperatures, to 80–90 percent confluence in 24-well plates for virus assay work.

(iii) Decant the medium from the required number of 24-well plates of each cell line, and inoculate four replicate wells per cell line with .10 ml per well of each processed sample. When all wells have been inoculated, tilt plates to spread the inocula evenly. Incubate inoculated plates for 1 hour at 15 °C. for sample contact. After the 1 hour con-

tact add cell culture medium. Medium shall be buffered or cells incubated so that a pH between 7.4 and 7.8 is maintained. All cell culture assays shall be incubated, without overlays, at 15 °C. for 21 days.

(4) *Virus identification by serological methods.* All cell cultures showing cytopathic effects (CPE) must be subcultured onto fresh cell cultures. If CPE is observed, determine the presence and identity the virus by serum neutralization, dot blot, enzyme-linked immunosorbent assay, or other equivalent serological technique.

(f) Information concerning the importation requirements of this section and application requirements for designation as a certifying official for purposes of this section may be obtained by contacting: U.S. Department of the Interior, U.S. Fish and Wildlife Service, Division of Fish Hatcheries (820 Arlington Square), 1849 C Street, NW., Washington, DC 20240. Telephone 703–358–1878.

(g) The information collection requirements contained in this part have been approved by the Office of Management and Budget under 44 U.S.C. 3501 *et seq.* and assigned clearance number 1018–0078. The information is being collected to inform U.S. Customs and USFWS inspectors of the contents, origin, routing, and destination of fish and eggs shipments and to certify that the fish lots were inspected for listed pathogens. The information will be used to protect the health of the fishery resource. Response is required to obtain a benefit.

[58 FR 58979, Nov. 5, 1993, as amended at 65 FR 37063, June 13, 2000; 67 FR 62203, Oct. 4, 2002; 72 FR 37469, July 10, 2007; 72 FR 59035, Oct. 18, 2007]

§ 16.14 Importation of live amphibians or their eggs.

Upon the filing of a written declaration with the District Director of Customs at the port of entry as required under § 14.61, all species of live amphibians or their eggs may be imported, transported, and possessed in captivity, without a permit, for scientific, medical, education, exhibition, or propagating purposes, but no such live amphibians or any progeny or eggs thereof may be released into the wild except by

the State wildlife conservation agency having jurisdiction over the area of release or by persons having prior written permission for release from such agency.

§ 16.15 Importation of live reptiles or their eggs.

(a) The importation, transportation, or acquisition is prohibited of any live specimen or egg of the brown tree snake (*Boiga irregularis*): Provided, that the Director shall issue permits authorizing the importation, transportation, and possession of such live snakes or viable eggs under the terms and conditions set forth in § 16.22.

(b) Upon the filing of a written declaration with the District Director of Customs at the port of entry as required under § 14.61, all other species of live reptiles or their eggs may be imported, transported, and possessed in captivity, without a permit, for scientific, medical, educational, exhibitional or propagating purposes, but no such live reptiles or any progeny or eggs thereof may be released into the wild except by the State wildlife conservation agency having jurisdiction over the area of release or by persons having prior written permission for release from such agency.

[55 FR 17441, Apr. 25, 1990]

Subpart C—Permits

§ 16.22 Injurious wildlife permits.

The Director may, upon receipt of an application and in accordance with the issuance criteria of this section, issue a permit authorizing the importation into or shipment between the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, or any possession of the United States of injurious wildlife (See subpart B of this part) for zoological, educational, medical, or scientific purposes.

(a) *Application requirements.* Submit applications for permits to import, transport or acquire injurious wildlife for such purposes to the Director, U.S. Fish and Wildlife Service, (Attention: Office of Management Authority), 4401 N. Fairfax Drive, Room 700, Arlington,

VA 22203. Submit applications in writing on a Federal Fish and Wildlife License/Permit application (Form 3-200) and attach all of the following information:

(1) The number of specimens and the common and scientific names (genus and species) of each species of live wildlife proposed to be imported or otherwise acquired, transported and possessed;

(2) The purpose of such importation or other acquisition, transportation and possession;

(3) The address of the premises where such live wildlife will be kept in captivity;

(4) A statement of the applicant's qualifications and previous experience in caring for and handling captive wildlife.

(b) *Additional permit conditions.* In addition to the general conditions set forth in part 13 of this subchapter B, permits to import or ship injurious wildlife for zoological, educational, medical, or scientific purposes shall be subject to the following conditions:

(1) All live wildlife acquired under permit and all progeny thereof, must be confined in the approved facilities on the premises authorized in the permit.

(2) No live wildlife, acquired under permit, or any eggs or progeny thereof, may be sold, donated, traded, loaned, or transferred to any other person unless such person has a permit issued by the Director under § 16.22 authorizing him to acquire and possess such wildlife or the eggs or progeny thereof.

(3) Permittees shall notify the nearest Special Agent-in-Charge (see § 10.22 of this chapter) by telephone or other expedient means within 24 hours following the escape of any wildlife imported or transported under authority of a permit issued under this section, or the escape of any progeny of such wildlife, unless otherwise specifically exempted by terms of the permit.

(c) *Issuance criteria.* The Director shall consider the following in determining whether to issue a permit to import or ship injurious wildlife for zoological, educational, medical, or scientific purposes:

(1) Whether the wildlife is being imported or otherwise acquired for a bona