CHAPTER 04 – PESTICIDES AND CHEMICAL CONTAMINANTS

SUBJECT:	**CHEMOTHERAPEUTICS IN AQUACULTURE SEAFOOD**
IMPLEMENTATION DATE:	UPON RECEIPT
Product Codes:	See Table 1
Product/Assignment Codes:	04018 Chemotherapeutics in Seafood

FIELD REPORTING REQUIREMENTS:

A. There are no hard copy reports for this Compliance Program.

B. Resources for completion of this Compliance Program can be found in the current ORA Field Workplan under PAC 04018, Chemotherapeutics in Seafood Compliance Program.

C. FACTS/OASIS reporting for domestic and import sample collections and for domestic and import sample analyses:
   1. PAC: 04018
   2. PAF: ANT

Table 1 – Product Codes Description for Different Species

INDUSTRY CODES: 16

PRODUCT CODES:

Product	**Code**
Crab	16J[][]01
Crayfish, Aquaculture Harvested Fishery/Seafood Products	16X[][]20
Crayfish (Crawfish), Fresh Water	16J[][]02
Eel	16A[][]15
Eel, Aquaculture Products	16X[][]45
Salmon, all, Aquaculture Harvested Fishery/Seafood Products	16X[][]03
Salmon (Humpback, Silver, King, Sockeye, etc.)	16A[][]32
Shrimp and Prawns, Aquaculture Harvested Fishery/Seafood Products	16X[][]21
Shrimp and Prawns	16J[][]05
Tilapia, Aquaculture Harvested Fishery/Seafood Products	16X[][]06
Tilapia	16A[][]58
Trout, all, Aquaculture Harvested Fishery/Seafood Products	16X[][]05
Trout (Rainbow, Brook, Brown, Char, Steelhead, etc.)	16A[][]44
Frogs, Aquaculture Harvested Fishery/Seafood Products	16X[][]42
Frog Legs, Other Aquatic Species	16M[][]01
Fish, N.E.C.[1]	16A[][]99
Aquaculture Harvested Fishery/Seafood Products, N.E.C.	16X[][]99
Crustaceans, N.E.C.	16J[][] 99
Barramundi	16A[][]85
Bass, Salt or Brackish Water (Stripe Bass, white Perch, Black Bass, etc.)	16A[][]04
Bass, Hybrid Striped, Aquaculture Harvested Fishery/Seafood Products	16X[][]01
Bream	16A[][]80
Carp	16A[][]09
Char	16X[][]99
Cobia	16A[][]94

Product	**Code**
Cobia, Aquaculture Harvested Fishery/Seafood Products	16X[][]47
Croaker	16A[][]13
Dace	16A[][]57
Dace, Aquaculture Harvested Fishery/Seafood Products	16X[][]44
Drum, Totoaba	16A[][]68
Grouper	16A[][]17
Lobster	16J[][]04
Langostino, lingostino	16J[][]07
Milkfish	16A[][]53
Mudfish	16A[][]64
Mullet	16A[][]23
Perch, Freshwater (Yellow Lake)	16A[][]26
Pompano (Permit, Pompanito)	16A[][]29
Pompano, (Permit, Pompanito) Aquaculture Harvested Fishery/Seafood Products	16X[][]07
Rohu	16X[][]99
Snapper (Red, Gray, Malabar, etc.)	16A[][]37
Sturgeon (River and Sea)	16A[][]40
Sturgeon, Aquaculture Harvested Fishery/Seafood Products	16X[][]04
Turbot	16A[][]46
Yellowtail, Amberjack	16A[][]79
Zander	16X[][]99

[1] N.E.C.-Not Elsewhere Classified

Note: 16A – Wild Caught Seafood Products

16X – Aquaculture Seafood Products

16J- Wild Caught Crustaceans Products

16M – Other Aquatic Species Products

For any other species, refer to Fish and Fishery products Hazard and Controls Guidance: CHAPTER 3 Potential Species-Related Hazards

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PART I – BACKGROUND

Over the past twenty years, there has been an extensive commercialization and an increased consumption rate of seafood products originating from aquaculture operations.

Aquaculture, also known as fish and shellfish farming, refers to the breeding, rearing and harvesting of aquatic food animals under environmentally controlled conditions with some form of intervention to enhance production, such as regular stocking, feeding, prevention and treatment of diseases, protection from predators, etc. Aquaculture activities are conducted in all types of water environments; freshwater, coastal and marine, inland ponds, tanks, reservoirs, rivers, lakes, estuaries, bays, fjords, and open sea.

In 2016, the world aquaculture production accounted for almost half of all fish for human food. This share is projected to rise to 62% by 2030 as catches from wild capture fisheries level off and demands for seafood constantly increases. The worldwide aquaculture production for human consumption has grown from 6.7 million metric tons in 1984 to 80 million metric tons ($ 231.6 billion value) in 2016. Fifteen main producer countries accounted for 92.5% of all farmed food fish. Asia accounted for 89% of world aquaculture production by volume and this continued to be dominated by China with around 62% of the global production. Other major producers are India, Vietnam, Indonesia, Bangladesh, Thailand, Chile, Egypt, Norway, Myanmar, Philippines, Japan, Brazil and Malaysia. (FAO: Fishery & Aquaculture Department in The State of World Fisheries and Aquaculture, 2018).

Aquaculture production is vulnerable to diseases and changing environmental conditions. Disease outbreaks significantly affect aquaculture production and trade. In recent years, farming areas in Asia and South America have been affected by diseases and problems from viruses, bacteria, fungi, parasites and other undiagnosed and emerging pathogens. The use of veterinary drugs in animal production is necessary to treat and control diseases; however, this use may result in the residues found in products for human consumption. The use of unapproved drugs and the misuse of FDA approved new animal drugs in aquaculture species is a concern. These uses may increase due to limited availability of treatment options, specifically for new emerging diseases. Veterinary drugs can also be administered to improve the production, e.g., to enhance feed efficiency and rate of weight gain. To protect consumers, it is important to ensure that both imported and domestic aquaculture seafood products are free from potentially harmful drug residues. Residues of animal drugs, the parent compound or its metabolite, in food may cause acute or chronic effects.

An acute response can occur from hypersensitivity or allergic reaction to the drug. For example, drug residue responses were reported in Spain, France, and China, where people became seriously ill after consumption of liver contaminated with clenbuterol residues. Other type of drugs, such as penicillin and, cephalosporins can cause allergic response at low doses. Chronic, long-term effects are difficult to detect. Effects from consumption of food contaminated with drug residues for a prolonged period of time are typically underreported to FDA or it's difficult to identify the cause of illness. However, the long-term hazards include extremely serious conditions. For example, nitrofurans and triphenylmethane dyes (malachite green, crystal (gentian) violet) and/or their metabolites are considered to be carcinogenic and genotoxic, and chloramphenicol has been implicated as the causative agent in several cases of fatal aplastic anemia. Sulfonamides used at subtherapeutic and therapeutic concentrations in

food-animal production revealed issues with carcinogenic and mutagenic potential and thyroid toxicity.

Antimicrobial use in aquaculture production may also contribute to microbial responses and antimicrobial resistance in bacteria that may be transferred to humans. Antibiotic resistance is a global problem found in human and animal environments inadequate oversight and inappropriate use in all areas of human and animal medicine. Microbial responses of drug residues can affect human intestinal flora diminishing the activity of intestinal bacteria. Also, antibiotic residues can develop transient resistant microorganisms and zoonotic spread of pathogens to humans. For example, the unapproved use of fluoroquinolones, such as ciprofloxacin and enrofloxacin, poses risk of antimicrobial resistance in bacteria and the related serious human health consequences as untreatable infections due to lack of drug effectiveness in treatment.

Improper use of chemical compounds in global aquaculture production is a concern of FDA, due to the significant safety impact on US consumers.

PART II - MPLEMENTATION

I. Objective

To sample and analyze identified imported and domestic aquaculture seafood products and processed crab to determine the presence and amount of:

a) unapproved chemical compounds and,
b) unapproved new animal drugs such as antibiotics or anti-fungal agents used for therapeutic and non-therapeutic purposes.
c) approved drug residue is at or above level exceeding the established tolerance level.

To take appropriate regulatory actions against aquaculture seafood products that are not in compliance.

II. Program Management Instructions

The Federal Food, Drug and Cosmetic Act (The Act) and FDA regulations apply equally to aquaculture seafood products produced in the United States (US) and aquaculture seafood products produced in other countries and imported into the US for commercial distribution. This compliance program establishes the collection and testing for residues of new animal drugs and unapproved animal drugs and chemical compounds in samples of aquaculture seafood products and processed crabmeat. Any samples found to contain illegal residues may lead to follow-up investigations at suspected firms and regulatory action.

CFSAN/ Office of Compliance/Division of Field Programs and Guidance will issue the Sample Collection Operations Planning Effort (SCOPE) that identifies the domestic and import aquaculture seafood species prior to the start of each Fiscal Year. SCOPE (Sample Collection Operation Planning Effort (SCOPE) (sharepoint.com)) is located through links on the CFSAN/Office of Compliance intranet website. Information about analyzing laboratories is provided in the SCOPE. It is imperative that the SCOPE information be followed to ensure proper sample collections.

A. Domestic

During sample collection of domestic products, if the investigator suspects that an unapproved new animal drug has been used on the farm or during transport, evidence of the intended use should be obtained at the point of sample collection and relayed to CVM contact. CVM may issue assignments to: i) document the use of the unapproved new animal drugs for possible enforcement action and, ii) follow-up on reports of positive drug residues when detected.

If the presence of residues of an unapproved drug or residues of approved drug at or above level exceeding the established tolerance level is confirmed by the analysis of a domestic sample, the divisions inform CFSAN and CVM to conduct a follow- up investigation.

B. Imports

Sampling of imported aquaculture seafood products are identified and directed in SCOPE. If the presence of residues of an unapproved drug equal to or above the Target Testing Level (TTL)/Regulatory Action Level (RAL) or residues of approved drug equal to or above the level exceeding the established tolerance level (TL) is confirmed by the analysis of an import sample, the division should detain the entry and proceed with the detention and hearing process. In addition, confirmation of the presence of a drug residue by sample analysis may result in recommendation for addition to an Import Alert for Detention Without Physical Examination (DWPE) of the combination of foreign processor and product or revision of existing Import Alert.

III. Program Interaction

- Illegal Drug Residues In Meat, Poultry, Seafood, And Other Animal Derived Foods Compliance Program 7371.006: Follow up the instructions to conduct on- farm investigations or investigations at a veterinarian or any other involved parties for drug residues from domestically produced product.
- Import Seafood Products Compliance Program, 7303.844: Use as a guide to conduct follow-up investigations at importers.
- Seafood Processor Inspection Program - Domestic and Foreign Facilities Compliance Program, 7303.842: Use for aquaculture seafood concerns other than chemotherapeutic agents.
- Aquaculture seafood products are also collected for Dioxin analysis as part of Pesticides and Industrial Chemicals in Food - Domestic and Imported Compliance Program (7304.004A). Divisions may coordinate sample collections as appropriate.

PART III - INSPECTIONAL

I. Operations

Collecting divisions: refer to ORA Field Workplan and the domestic and import Sample Collection Operation Planning Effort (SCOPE) issued by CFSAN before the start of each fiscal year for the specific numbers and the species of seafood to be collected and countries of interest. SCOPE is located at: Sample Collection Operation Planning Effort (SCOPE) (sharepoint.com)

Note: Only those products listed in the SCOPE should be collected.

A. Inspections

Resource for inspection has not been planned under this compliance program.

B. Investigations

1. Domestic Samples

During domestic sample collection at the aquaculture farm if the investigator suspects the use of unapproved new animal drugs on the farm or during transport, evidence of the intended use should be obtained, at the point of sample collection. The division should notify CVM for further follow-up investigation and a possible enforcement action.

2. Import Samples

For import samples, if an unapproved drug residues equal to or above the Target Testing Level (TTL)/Regulatory Action Level (RAL) or residues of approved drug equal to or above the level exceeding the established tolerance level (TL) are confirmed by sample analysis, the divisions's compliance branch should detain the entry and proceed with the detention and hearing process. In addition, confirmation of the presence of an unapproved drug residue equal to or above TTL/RAL or approved drug residue equal to or above TL by sample analysis, should result in a recommendation for addition to Detention Without Physical Examination (DWPE) on an applicable Import Alert for the combination of product and foreign processor. This recommendation should besent via Compliance Management Services (CMS) to the Division of Import Operations (DIO), Import Compliance Branch (ICB). DIO will forward the recommendation to CFSAN for assessment and evaluation. CFSAN will then communicate the conclusion of the evaluation to DIO for revision of the applicable Import Alert.

C. Sample Collections

1. Sampling Instruction

a) General

(1) Samples should be collected from the largest size of product's line/lot (as identified by a manufacturer, importer, or owner of the goods) available. If the line is composed of multiple lots collect samples across different lots.
(2) Samples of the smallest unit packages (with the weight closest to the sub needed) within the product's lot/line should be collected, whenever possible.
(3) Raw, unprocessed, breaded, seasoned, cooked, in sauce, in oil, fresh, or frozen product is acceptable for collection, unless otherwise specified in the SCOPE. ***Canned product, except for canned meat of crab, should not be collected***
(4) **Do not collect smoked and dried products**
(5) Frozen, refrigerated, pasteurized, canned meat of crab (typically in 6 oz cans), cut crab, soft shell crab, crab claws, cut crab pieces and crab legs should be collected. ***Whole crab should not be collected.***
(6) Whole crayfish is acceptable for collection.
(7) **Do not use black or blue Sharpie markers** when labeling aquaculture chemotherapeutic samples to avoid contamination of sample with marker ink. As an alternative, the investigator can use a red pen or stick-on labels.
(8) When collecting any fresh product, the investigator should coordinate with the assigned laboratory so that the applicable timeframes are met.
(9) For the purposes of shipping samples, samples of fresh seafood product can be frozen, however if samples are being shipped fresh, they should be shipped the same day as collection.

(b) Domestic Sample Collection

Refer to the current SCOPE domestic section for the number of each identified product to be collected.

All products **must be aquaculture, farmed raised** except for processed crab and crayfish. Processed crab and crayfish from either aquaculture operation or wild caught can be collected. Make sure that crab and crayfish are domestic origin and not mixed with imported product.

It is recommended to collect domestic samples at aquaculture farm or at the point of harvest (crab and crayfish). **The domestic farmer/grower or harvester should be identified in the Collection Report.** Domestic aquaculture product samples may also be collected from processors or wholesalers provided that the domestic farmer/grower can be identified and is reported in the Collection Report. Prior to collection, review the firm's inspection history or call the firm to verify that the product the firm processes or holds is domestic origin.

If the sample is fresh, the investigator should coordinate with the lab prior to shipment any fresh seafood that is on hold pending completion of analysis. In addition, annotate on the FDA 525 and use the appropriate sample flag on the Collection Report.

Do not collect Domestic Import (DI) samples to meet domestic sample collection obligations without consulting with CFSAN.

(c) Import Sample Collection

Refer to the current SCOPE for the number of import samples needed to collect of each product and countries of interest. Import SCOPE tracker is located at:

Sample Collection Operation Planning Effort (SCOPE) (sharepoint.com)

Imported seafood may not always be identified on labeling or in shipping documents as "aquaculture or farm raised." It should be assumed that barramundi, carp, cobia, dace, eel, frog legs, milkfish, pompano, shrimp/prawns, salmon, trout and tilapia are farm raised, unless accompanying paperwork or labeling clearly states "wild caught." Collect finfish species listed in the current FY SCOPE. Processed crab and crayfish may be collected regardless of whether it is "wild caught" or farm-raised.

If the sample is fresh, the investigator should notify the analyzing lab that fresh seafood is on hold pending completion of analysis by annotating the FDA 525 or the outside of the sample container and using the appropriate Problem Area Flag (PAF) on the Collection Report. When collected as a domestic import sample, the collector must include the entry number in the remarks section of the FACTS Sample Collection Report.

- **Import obligations may be met by collecting Domestic Import (DI) samples only if the division can identify the foreign processor/shipper, and the country of origin.**

- **The investigator should not select a product for sample collection when the combination of the foreign processor and product are already subject to DWPE due to the presence of unapproved drug residues.**

- **Avoid collecting multiple samples of one type of product from the same foreign processor in a short period of time, unless otherwise instructed.**

The import collecting divisions are encouraged to work with their Division Import Activities Liaisons (DIALs) to adjust the May-Proceed rates for those products that are required for collection by their division. The DIALs will work with the Division of System Solutions (DSS) /Import Systems Branch (ISB) to adjust the May-Proceed rates accordingly.

Audit/Certification sample is collected to verify analytical results provided by a certificate of analysis or private laboratory analysis that purports to show a product complies with the FD&C Act and/or regulations. Detail instructions about audit are provided in IOM 4.1.4.12.

(d) Sample Size

Domestic Samples

Item	Sample
Salmon Tilapia Trout Other: Arctic Char, Barramundi, Bass Hybrid Striped, Cobia, Carp, Drum, Eel, Grouper, Snapper, Yellow Croaker, Pompano, Milkfish, Mudfish, Mullet, Bream, Sturgeon, Perch, Turbot, Yellowtail	Each fresh, frozen, breaded or seasoned fish product's sample collected should consist of 12 subsamples, minimum 454 grams (1 lb.) per subsample, a total 5.4kg (12 lbs.) of product per sample. **Collect a separate 702(b) sample.** If the individual **package unit is larger than 454 grams (1 lb.),** collect one package unit per subsample. If the individual product **package unit is less than 454 grams (1 lb.)**, collect an adequate number of package units so that the total amount collected per subsample equals the minimum specified. ***Note: In situations the cost of indicated sample size(s) is/are prohibitive, the following alternate sampling scheme may be applied:*** For individual package size **larger than 1.35 kg (3lbs)** • Collect 6 subsamples plus a separate 702(b) portion of any fishery product listed here.

Item	Sample
Shrimp/Prawn	Each fresh or frozen sample collected should consist of twelve (12) subsamples, equaling a minimum of 454 grams (1 lb.) per subsample for a total product sample weight of 5.4 kg (12 lbs.). If the sampled product is whole, in-shell, or breaded, a minimum of 680 grams (1.5 lbs.) per subsample should be collected for a total product sample weight of 8.2 kg (18 lbs.). **Collect a separate 702(b) sample.** **Note: For individual units less than 454 grams (1.0 lb.):** If the individual product package size is less than 454 grams (1 lb.), collect an adequate number of individual product package units per subsample to equal a minimum of 454 grams (1 lb.) for fresh or frozen product or 680 grams (1.5 lbs.) for whole, in-shell or breaded product. **Note: For individual units larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.):** If the individual product package unit size is larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.), collect one individual package unit per subsample. **Note: For individual units larger than 1.35 kg (3.0 lbs.) only:** If the units must be sampled and shipped intact, collect 6 subsamples (individual product package units). Alternatively, subsamples of at least 454 grams (1 lb.) may be broken/sawed off (keep frozen) from each of 12 individual package units, and the twelve (12) 454 grams subsamples shipped to the analyzing lab. If sampling from bulk, collect using aseptic technique (refer to IOM 4.3.6).

Item	Sample
Processed Crab, Crayfish, Lobster and Langostino	Each sample should consist of twelve (12) subsamples, minimum 454 grams (1 lb.) per subsample, a total 5.4 kg (12 lbs.) of product per sample. If the sampled product is whole, in-shell or is cut pieces still in shell, a minimum of 680 grams (1.5 lbs.) per subsample should be collected, a total 8.2 kg (18 lbs.) of product per sample. **Collect a separate 702(b) sample.** **Note: For individual units less than 454 grams (1.0 lb.) only:** If the individual product package unit size is less than 454 grams (1 lb.), collect an adequate number of individual product package units per subsample to equal a minimum of 454 grams (1 lb.) or 680 grams (1.5 lbs.) for whole, in-shell product per subsample. **Note: For individual units larger than 454 grams (1 lb.):** If the individual product package unit size is larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.), collect one package unit per subsample. **Note: For individual units larger than 1.35 kg (3.0 lbs.) only:** If the units must be sampled and shipped intact, collect 6 subsamples (individual product package units). Alternatively, subsamples of at least 454 grams (1 lb.) may be broken/sawed off (keep frozen) from each of 12 individual package units, and the twelve (12) 454 grams subsamples shipped to the analyzing lab. If sampling from bulk, collect using aseptic technique (refer to IOM 4.3.6).

Import and Domestic Import Sample Size

Item	Sample
Frog legs Eel Salmon Tilapia Trout Other: Arctic Char, Barramundi, Bass Hybrid Striped, Carp, Cobia, Dace, Drum, Grouper, Snapper, Yellow Croaker, Pompano, Milkfish, Mudfish, Mullet, Bream, Sturgeon, Perch, Turbot, Yellowtail	For finfish, each fresh, frozen, breaded, or seasoned product's sample collected should consisit of twelve (12) sumbsamples, equaling a minimum of 454 grams (l lb.) per subsamples for a total product sample weight of 5.4 kg (12 lbs.) For frog legs, twelve (12) subsamples equaling a minimum of – 680 grams (1.5 lbs.) per subsample for a total product sample weight of 8.2 kg (18 lbs.) should be collected. Collect one individual package unit per subsample: if the individual product package **unit is larger than 454 grams (1 lb.) for any finfish type;** OR if the individual product package **unit is larger than 680 grams (1.5 lbs) for frog legs**. Collect an adequate number of individual package units so that the total amount collected per subsample equals the minimum specified weight for each fish type: if the individual product package **unit is less than 454 grams (1 lb.) for any finfish type;** OR if the individual package **unit is less than 680 grams (1.5 lbs) for frog legs**. ***Note: In situations the cost of indicated sample size(s) is/are prohibitive, the following alternate sampling scheme may be applied:*** For individual package size **larger than 1.35 kg (3 lbs)** • Collect 6 subsamples of any fishery product listed here.

Item	Sample
Shrimp/Prawn	Each fresh or frozen sample collected should consist of twelve (12) subsamples, equaling a minimum of 454 grams (1 lb.) per subsample for a total product sample weight of 5.4 kg (12 lbs.). If the sampled product is whole, in-shell, or breaded, a minimum of 680 grams (1.5 lbs.) per subsample should be collected for a total product sample weight of 8.2 kg (18 lbs.). **Note: For individual units less than 454 grams (1.0 lb.):** If the individual product package unit is less than 454 grams (1 lb.), collect an adequate number of individual product package units per subsample to equal a minimum 454 grams (1 lb.) of fresh or frozen, or 680 grams (1.5 lbs.) of whole, in-shell or breaded shrimp product. **Note: For individual units larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.):** If the individual product package unit size is larger than 454 grams (1 lb.), and less than or equal to 1.35 kg (3 lbs.), collect one package unit per subsample. **Note: For individual units larger than 1.35 kg (3.0 lb.):** If the units must be sampled and shipped intact, collect 6 subsamples (individual product package units). Alternatively, subsamples of at least 454 grams (1 lb.) may be broken/sawed off (keep frozen) from each of 12 individual product package units. If sampling from bulk, collect using aseptic technique (refer to IOM 4.3.6).

Item	Sample
Processed Crab, Crayfish, Lobster and Langostino	Each sample should consist of twelve (12) subsamples, minimum 454 grams (1 lb.) per subsample, for a total of 5.4 kg (12 lbs.) of product. If the product is whole in-shell or cut crab pieces still in shell, a minimum of 680 grams (1.5 lbs.) should be collected per subsample, for a total of 8.2 kg (18 lbs.) of product per sample. **Note: For individual units less than 454 grams (1 lb.):** If the unit size is less than 454 grams (1 lb.), collect an adequate number of individual product package units per subsample to equal a minimum of 454 grams (1 lb.) for processed product and 680 grams (1.5 lbs.) for whole, in-shell product. **Note: For individual units larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.):** If the individual product package unit size is larger than 454 grams (1 lb.) and less than or equal to 1.35 kg (3 lbs.), collect one individual package unit per subsample. **Note: For individual units larger than 1.35 kg (3.0 lbs) only:** If the units must be sampled and shipped intact, collect 6 subsamples (individual product package units). Alternatively, subsamples of at least 454 grams (1 lb.) may be broken/sawed off (keep frozen) from each of 12 individual package units. If sampling from bulk, collect aseptically (refer to IOM 4.3.6).

2. Shipping Instructions

Shipping instructions to maintain the integrity of frozen and refrigerated samples and the procedure to notify the receiving laboratory can be found in IOM, Sections 4.7.3.5 (frozen samples) and 4.7.3.6 (refrigerated samples). Samples should be packaged with the appropriate refrigerant and shipped for their arrival at the laboratory no later than Thursday of each week. Do not ship samples on Fridays for Saturday delivery.

Samples should only be shipped to the laboratory listed in the current Lab Servicing Table for Chemotherapeutics.

D. Import Activities

N/A

E. Other

N/A

II. Reporting

Report resources utilized for sample collection using the following Program Assignment Codes (PACs) and Problem Area Flags (PAF):

PAC	PAF	PAF Description
04018	ANT	Antibiotic Residues

Audit samples should be recorded under the same PAC codes as surveillance samples and can apply towards the completion of applicable Work Plan and/or Performance goals.

PART IV - ANALYTICAL

I. Analyzing Laboratories

Refer to the current Lab Servicing Table Dashboard for ANT to determine the analyzing laboratories.

II. Analyses to be Conducted

Refer to the current FY SCOPE to determine the analyses to be conducted.

II. Methodology

A. Sample Preparation and Methods

1. General Instructions

The chemical compounds identified in the following methods to be tested in seafood species listed in this Compliance Program have human health concerns and might pose a food safety risk to the US consumers. The determinative method will identify and quantify the amount of the compound and the confirmation method will validate findings of the determinative method (if applicable).

Report all analytical results in FACTS. Retain the remaining portion of each subsample when classified as Lab Class 3 (Adverse Finding).

2. Analytical Protocol

Prepare a composite of all subsamples, unless otherwise noted for specific drug residue testing that requires individual subsample analysis.

Whenever the analytical result of the residue reported from the determinative (quantitative) method is equal to or above the level referenced below for each drug residue (under **C. Target Testing Level (TTL)**), a confirmatory method must be run to confirm the identity of the residue in the sample of specific species (where applicable).

If the determinative method is also a confirmatory method (e.g., LC-MS/MS) and the criteria are met to confirm the identity of the residue, then a second confirmatory analysis is not required.ORWhenever the analytical result of the residue identified from screening method is above the threshold level associated with the TTL referenced below for each drug residue (under C. Target Testing Level (TTL)), a determinative method must be run to provide quantitative analysis of the amount of residue in the sample.

3. Sample Preparation

Homogenize only edible portion. Generally, the edible portion comprise:
(a) For finfish – muscle or muscle with skin in natural proportions (as received in the laboratory)
(b) For shrimp or prawns - soft tissue including mid-intestinal gland, excluding shell
(c) For crab, crayfish, lobster, and langostino- soft tissue excluding shell
(d) For soft shell crab-the entire crab including shell (as received in the laboratory)
(e) For frog-leg muscle excluding skin and bone

Thoroughly remove bones, shell, any breading, wipe or blot excessive sauce, oil or seasoning from the edible portion before analysis.It is suggested that laboratories homogenize sample by grinding with dry ice (reference: Bunch, E.A., Altwein, D.M., Johnson, L.E. Farley, J.R., and Hammersmith, A.A. (1995) Homogenous Sample Preparation of Raw Shrimp with the Aid of Dry Ice. *J. AOAC Int*. 78, 883-887).

Divide the prepared (processed with dry ice) composite or individual subsample in half. Use half of the prepared sample for analysis and retain the other half of the prepared sample in a freezer as a reserve.

For domestic samples, the 702(b) protions from each of the subsample collected should remain untocuched.

For Chloramphenicol and Nitrofurans:

(1) All chloramphenicol analyses are to be run on an individual subsample basis.
(2) All nitrofurans analyses are to be run on an individual subsample basis.

The confirmation of chloramphenicol or nitrofurans in a single subsample is sufficient to establish the residue presence.

The laboratory is to continue analyzing individual subsamples until either chloramphenicol or nitrofuran residue is confirmed in a subsample portion, or a total of 12 negative subsample portions are completed. Alternately, a two-subsample composite may be prepared provided that the lab has a documented study that demonstrates adequate sensitivity and has **prior approval from ORS**. If the analysis of the two-subsample composite indicates the presence of a target analyte at 40% of the Target Testing Level (TTL), the two subsamples must be analyzed individually.

If 12 subsamples are collected (3 lbs. or less per unit), select at random approximately 100 grams of product (chipped from block if frozen) from each subsample. If 6 subsamples (>3 lbs. units) were collected, analyze individually each of two (2) approximately100 g portions, taking the portions from opposite ends of the subsample.

For All Other Drug Residues:

Prepare one composite by combining portions of all subsamples.

If 12 subsamples are collected (3 lbs. or less), select an equal representative sample, randomly or chipped from block, (refer to the laboratory SOPs) from each subsample.

If 6 subsamples (>3 lbs. units) were collected, select an equal representative sample from each opposite end of the subsample for frozen blocks (refer to the laboratory SOPs). For bags of product, randomly select two equal representative samples from the bag for the composite (refer to the laboratory SOPs).

B. Methodology

Refer to the current FY SCOPE for priority of residues to be tested for each species. It is very important that when results are entered into FACTS, the PAF = ANT must be used.

In the following table, hyperlinks when available have been provided for methods. Where there is no hyperlink and when a laboratory does not have access to the referenced methods, they should contact Connie Drake , ORA/ORS/FFSS at 870-543-4046 or e-mail Connie.Drake@fda.hhs.gov for a copy of the methods.

Method LIB#	Method Name	Drug Class	Analytes
LIB 4597	Quantitation of **Chloramphenicol** and **Nitrofuran** Metabolites in Aquaculture Products Using Microwave-Assisted Derivatization, Automated Solid-Phase Extraction and LC-MS/MS (catfish, crawfish, frog legs, crab, and shrimp)	Nitrofuran and Chloramphenicol	Chloramphenicol Semicarbazide (SC) 3-amino-2-oxazolidinone (AOZ) 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) 1-aminohydantoin (AHD)
LIB 4682	Modifications to LIB 4597 for the analysis of nitrofuran metabolites and chloramphenicol in aquaculture products using LC-MS/MS	Nitrofuran and Chloramphenicol	Chloramphenicol Semicarbazide (SEM) 3-amino-2-oxazolidinone (AOZ) 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) 1-aminohydantoin (AHD) 3,5-dinitrosaliscylic acid hydrazide (DSH)

Method LIB#	Method Name	Drug Class	Analytes
LIB 4473	Determination of Chloramphenicol in Shrimp, Crawfish, Crab, and Frog Legs Using High Throughput LC/MS/MS	Chloramphenicol	Chloramphenicol
LIB 4646	**Triphenylmethane Dye** Residue Analysis in Raw and Processed Aquaculture Products by AOAC Official Method of Analysis 2012.25	Triphenylmethane Dyes	Malachite Green Leucomalachite Green Crystal Violet Leucocrystal Violet Brilliant Green
LIB 4496	Liquid Chromatography-Tandem Mass Spectrometry Method for the Confirmation and Quantitative Analysis of **Avermectin** Residues in Salmon	Avermectins	Ivermectin Emamectin Abamectin Doramectin
LIB 4636	A Rapid Liquid Chromatography-Fluorescence Detection (UPLC/FLD) for the Quantitative Analysis of **Avermectin** Residues in Salmon and Trout	Avermectins	Ivermectin, Emamectin, Abamectin, Doramectin
LIB 4619/ LIB 4626	Development and Validation of a LC-MS/MS Method for the Determination of **Isoeugenol** in Finfish with Rapid Extraction and Simple Off- line Derivatization (Refer to methods for specific alteration)	Isoeugenol	Isoeugenol
LIB 4535	Analysis of **Stilbene** Residues in Aquacultured Finfish using LC-MS/MS	Stilbenes	Diethylstilbestrol Dienestrol Hexestrol

Method LIB#	Method Name	Drug Class	Analytes
LIB 4562/ LIB 4614	Analysis of **sulfonamides, trimethoprim, fluoroquinolones, quinolones, triphenylmethane dyes (and their leuco metabolites) and methyltestosterone** in fish and shrimp using liquid chromatography mass spectrometry	Multi-Class Residues	Sulfacetamide Sulfachloropyridazine Sulfadiazine Sulfadimethoxine Sulfadoxine Sulfaethoxypyridazine Sulfamerazine Sulfamethazine Sulfamethoxazole Sulfamethoxypyridazine Sulfamonomethoxine Sulfapyridine Sulfaquinoxaline Sulfathiazole Trimethoprim Ciprofloxacin Enrofloxacin Norfloxacin Sarafloxacin Danofloxacin Difloxacin Flumequine Nalidixic Acid Oxolinic Acid Methyltestosterone Malachite Green Leucomalachite Green Crystal Violet Leucocrystal Violet Brilliant Green Florfenicol Florfenicol Amine Thiamphenicol Chlortetracycline Tetracycline Oxytetracycline Mebendazole Mebendazole Amine Hydroxymebendazole

Method LIB#	Method Name	Drug Class	Analytes
LIB 4653A	Multi-class Veterinary Drug Residue Method for Aquaculture Products Using LC-MS/MS	Multi-Class Residues	Enrofloxacin Ciprofloxacin Danofloxacin Difloxacin Norfloxacin Ofloxacin Sarafloxacin Nalidixic Acid Oxolinic Acid Flumequine Lincomycin Erythromycin Doxycycline Tetracycline Oxytetracycline Chlortetracycline Sulfamethazine Sulfamerazine Sulfadimethoxine Sulfadiazine Sulfachloropyridazine Sulfaquinoxaline Sulfathiazole Sulfacetamide Sulfaethoxypyridazine Sulfamethoxazole Sulfamethoxypyridazine Sulfapyridine Sulfadoxine Sulfamonomethoxine Malachite Green Leucomalachite Green Crystal Violet Leucocrystal Violet Trimethoprim Hydroxy Mebendazole Mebendazole Amine Mebendazole Thiabendazole Florfenicol Amine Ampicillin Amoxicillin Cloxacillin Thiamphenicol Azamethiphos

C. Target Testing Level (TTL)/ Regulatory Action Level (RAL)

The following values are the current Target Testing Levels (TTL) or tolerance level (TL) for each chemotherapeutic agent. These levels are also considered as Regulatory Action Levels (RAL). However, TTL is not and should not be interpreted as a safe concentration or a tolerance level and it does not imply that an approval exists for that drug [21CFR530.3(g)].

Animal Drug Residue	**Target Testing Level or Tolerance Level (ppb)**
Chloramphenicol[1]	0.15
Nitrofurans[1] AOZ metabolite of Furazolidone AMOZ metabolite of Furaltadone SC metabolite of Nitrofurazone AHD metabolite of Nitrofurantoin DSH metabolite of Nifursol	 0.5 0.5 0.5 0.5 0.5
Ampicillin Amoxicillin Cloxacillin	10.0 10.0 10.0
Avermectins: Ivermectin Abamectin Doramectin	 10.0 10.0 10.0
Azamethiphos[5]	20.0
Emamectin[2]	100.0
Benzimidazoles: Sum of Mebendazole + Mebendazole Amine + Hydroxy Mebendazole Thiabendazole	 5.0 100.0
Erythromycin	100.0
Florfenicol Amine[3]	1,000.0
Fluoroquinolones: Sum of Enrofloxacin and Ciprofloxacin Danofloxacin Difloxacin Norfloxacin Ofloxacin Sarafloxacin	 5.0 5.0 5.0 5.0 5.0 5.0
Isoeugenol	200.0
Lincomycin	10.0
Methyltestosterone	0.8
Quinolones: Oxolinic Acid	 10.0

Animal Drug Residue	**Target Testing Level or Tolerance Level (ppb)**
Flumequine	10.0
Nalidixic Acid	10.0
Sulfonamides:	
Sulfamerazine	10.0
Sulfadiazine	10.0
Sulfachloropyridazine	10.0
Sulfathiazole	10.0
Sulfaquinoxaline	10.0
Sulfamethazine	10.0
Sulfadimethoxine	10.0
Sulfacetamide	10.0
Sulfadoxine	10.0
Sulfaethoxypyridazine	10.0
Sulfamethoxypyridazine	10.0
Sulfamethoxazole	10.0
Sulfapyridine	10.0
Sulfamonomethoxine	10.0
Stilbenes:	
Diethylstilbesterol	0.25
Dienesterol	0.25
Hexestrol	0.25
Tetracyclines:[4]	
Sum of Oxytetracycline + Tetracycline + Chlortetracycline	2,000.0
Thiamphenicol	1.0
Trimethoprim	10.0
Triphenylmethane Dyes:	
Sum of Malachite Green and Leucomalachite Green	0.5
Sum of Crystal Violet and Leucocrystal Violet	0.5
Brilliant Green	0.5

[1] Results of all five nitrofuran metabolites and chloramphenicol analysis have to be reported for individual subsamples of a sample.

[2] Import Tolerance of 100 ppb established for Emamectin B1a (the marker residue) in muscle with adhering skin in salmonids.

[3] Tolerance Level of 1.0 ppm (1000.0 ppb) established for Florfenicol Amine (marker residue for Florfenicol) in aquaculture species (salmonids, and other freshwater-reared finfish) (21CFR556.283(b)).

[4] Tolerance Level of 2.0 ppm (2000.0 ppb) established for the sum of residues of the tetracyclines including Tetracycline, Oxytetracycline, and Chlortetracycline in finfish and lobster (21CFR556.500(b)(1)).

[5] Improt Tolerance of 20 ppb established for Azamethiphos in muscle with adhering skin in salmonids.

D. Reporting

Enter all analytical results into FACTS using PAC 04018 and PAF ANT. The following criteria should be used for classification of samples;

Lab Class "1":

1. The sample contains no residue of unapproved animal drug,
   or
2. The sample contains residues of FDA approved drug that are below the tolerance level and Target Testing Level (TTL).

Lab Class "2":

1. The sample with residues below the tolerance level or TTL and technically classified as the Lab Class 2 should be reported as the Lab Class 1 in FACTS.

Lab Class "3":

1. The reported residue concentration of unapproved drug at or above TTL (listed in Part IV- Analytical, Section III.C),
   or
2. The reported residue concentration of FDA approved drug at or above the tolerance level.

PART V - REGULATORY/ADMINISTRATIVE STRATEGY

Samples must meet the following criteria to be submitted to DIO/CFSAN:

1. Sample was collected consistent with the instructions in Part III, Inspectional, A. Sample collection.
2. Sample preparation and analytical methods used are only those listed under Part IV, Sample Preparation, 3 and Methodology, B.
3. The presence and confirmation (if applicable) of the residue found in any sample is at a level greater than or equal to the Target Testing Level (unapproved drugs) or Tolerance Level (approved drugs) listed in Part IV, Target Testing Levels (TTL)/Regulatory Action Level (RAL), C.

One of the goals of this program is to obtain sufficient evidence to support broad-based enforcement strategies. Divisions should be aware of detention patterns that could indicate a prevalent problem with a particular product, a processor, an importer, a particular country or a specific region and notify CFSAN's Compliance Office when these situations arise. The Center intends to refocus its regulatory efforts as appropriate.

Import Compliance Actions:

If the drug residue violation is confirmed by the analysis of an import sample, the import division should refer to the appropriate Import Alert (IA) below for appropriate follow-up action, including submitting a recommendation for addition to an Import Alert red list or removal from an Import Alert green list to DIO's Import Compliance Branch (ICB) via CMS.

Unapproved Animal Drug Residues in All Aquaculture Seafood Products Import Alert #16-124

DETENTION WITHOUT PHYSICAL EXAMIATIONS OF AQUACULTURE SEAFOOD PRODUCTS DUE TO UNAPPROVED DRUGS

Chloramphenicol in Crustaceans (crab, crayfish, shrimp, lobster, langostino) Import Alert #16-127

DETENTION WITHOUT PHYSICAL EXAMINATION OF CRUSEACEANS DUE TO CHLORAMPHENICOL

Nitrofurans in All Seafood Products Import Alert #16-129

DETENTION WITHOUT PHYSICAL EXAMINATION OF SEAFOOD PRODUCTS DUE TO NITROFURANS

Unapproved Animal Drug Residues in Aquaculture Seafood Products (shrimp, dace, and eel) from China-countrywide Import Alert#16-131

DETENTION WITHOUT PHYSICAL EXAMINATION OF AQUACULTURED SHRIMP, DACE, AND EEL FROM CHINA- PRESENCE OF NEW ANIMAL DRUGS AND/OR UNSAFE FOOD ADDITIVES

Unapproved Animal Drug Residues in Aquaculture Shrimp and Prawns from Peninsular Malaysia Import Alert # 16-136

DETENTION WITHOUT PHYSICAL EXAMINATION OF AQUACULTURED SHRIMP AND PRAWNS FROM PENINSULAR MALAYSIA DUE TO PRESENCE OF DRUG RESIDUES FROM UNAPPROVED ANIMAL DRUGS OR THE PRESENCE OF UNSAFE FOOD ADDITIVES

The division should refer to any additional instructions in the Regulatory Procedures Manual, Chapter 9.

The home division should consider a follow-up HACCP Inspection, per the Import Seafood Products Compliance Program, 7303.844, at the importer whose products were found to contain illegal residues.

The foreign processing establishment that produced and offered for entry the seafood product adulterated with residues should be considered for a HACCP inspection during planning of foreign regulatory inspections.

Domestic Compliance Actions:

At this time, all positive domestic sample results should be discussed with CFSAN's Office of Compliance/Division of Enforcement who will coordinate regulatory follow-up with CVM.

If a drug residue is confirmed in domestic produced seafood, CVM will take the lead on providing direction to determine the cause of the residue. If a follow-up inspection is warranted, the Compliance Program, 7371.006, Illegal Drug Residue in Meat, Poultry, Seafood and Other Animal Derived Foods will be used to conduct investigations of producers and other involved parties as appropriate. All the residues of the drugs that are being tested are unapproved new animal drugs, therefore, CVM will consider enforcement action for any violative residues when the responsibility, jurisdiction and violation are all documented.

CVM General and Regulatory Contact: Marianne P Martinson, (240) 402-5624

PART VI REFERENCES, ATTACHMENTS, AND PROGRAM CONTACTS

A. References

FDA ORA Regulatory Procedures Manual (RPM)

FDA ORA Investigations Operation Manual (IOM)

B. Program Contacts

CFSAN

Program Contact:	Teja Patel	CFSAN, Office of Compliance Division of Field Programs and Guidance Program Assignment & Monitoring Branch, HFS-615 (240) 402-2339 Teja.Patel@fda.hhs.gov
Regulatory Contact:	DE Chemotherapeutics in Aquaculture Seafood Team	Chmotherapeutics in Aquaculture Seafood Team CFSAN, Office of Compliance Division of Enforcement CMS: CFSAN Food Adulteration Assessment Branch DEChemotherapeutics@fda.hhs.gov
Scientific Contact:	Barbara Montwill	CFSAN, Office of Food Safety Division of Seafood Safety Shellfish and Aquaculture Policy Branch, HFS-325 (240) 402-1426 barbara.montwill@fda.hhs.gov

CVM

Scientific Contact:	Julia Oriani	CVM, ONADE, Division of Human Food Safety, HFV-510 (240) 402-0788 julia.oriani@fda.hhs.gov

ORA

Technical Contact:	Wendy Andersen	ORA, Animal Drugs Research Center (303) 236-3074 wendy.andersen@fda.hhs.gov
Import Operations: Liaison	CFSAN/OIO	ORA, OEIO/Division of Import Operations Import Operations Branch ORAOEIODIOCFSANLiaisons@fda.hhs.gov
Domestic Investigations:	Rina Vora	ORA/OO/OHAFOW/DDHAFO/DHAFOB HFC-130 (561) 416-1065 EXT 1117 rina.vora@fda.hhs.gov
Scientific Contact:	Connie P Drake	ORA, ORS, Human and Animal Food Scientific Staff, HFR-SW500 (870) 370-4003 Connie.Drake@fda.hhs.gov

SCIENTIFIC METHOD ANALYSIS CONTACTS

CVM Contact	Julia Oriani	CVM/ONADE	240-402-0788
Avermectins/Emamectin	Christine Casey	ORA/DENLHAF	303-236-9630
Chloramphenicol/ Nitrofurans	Brian Veach	ORA/ARLHAF	870-543-4085
Isoeugenol	Lin Ye	ORA/ATLHAF	404-575-1536
Methyltestosterone	Sherri Turnipseed	ORA/ADRC	303-236-3072
Multi-class residue methods	Sherri Turnipseed Brian Veach	ORA/ADRC ORA/ARLHAF	303-236-3072 870-543-4085
Stilbenes	Wendy Andersen Christine Casey	ORA/ADRC ORA/DENLHAF	303-236-3074 303-236-9630
Triphenylmethane Dyes (Malachite Green/Crystal Violet/Brilliant Green)	Wendy Andersen	ORA/ADRC	303-236-3074

PART VII - CENTER RESPONSIBILITIES

The Office of Food Safety (OFS) will provide subject matter expertise in the maintenance and evaluation of the Compliance Program and provide guidance to the Office of Compliance with regard to program priorities, relevant evaluation questions, and recommended program changes. The Office of Compliance will lead the effort and work in conjunction with the Office of Food Safety (OFS) and CVM to prepare routine compliance program evaluations. Evaluation will be conducted on a periodic basis and outline the program office's current objectives, general and specific program evaluation questions, list recommendations for process improvement, and highlight data patterns and trends for better targeting and resource allocation. The Office of Compliance will make these evaluations available internally to FDA. In addition, the Office of Compliance will prepare an annual summary report of this compliance program which will be available at Compliance Program Summaries.