Monitoring Antibiotic Residues in Fish & Fishery Products

Design & Details of NRCP & PHT

-MPEDA
Residue Control Measures implemented by MPEDA at primary production level:

1. **National Residue Control Plan (NRCP) for aquaculture Products**

1. **Pre-Harvest Testing (PHT) of aquaculture products.**
NRCP – Role of Field Offices:

- Collection & dispatch of samples from the hatcheries/ farms / feed mills to the designated labs.
- Creating awareness among the farmers on the adverse effects due to usage of Antibiotics/VMPs in farming operations.
- Provide guidance/training to hatcheries to produce seeds without the usage of antibiotics.
- Investigation/surveillance of farms, hatcheries and processing plants, in case of non-compliant results.
IMPLEMENTATION OF NRCP

Field Offices of MPEDA (28):

- Regional Centers - 7
- Sub-Regional Centers - 5
- Satellite Centers - 2
  - Sample collection
  - Communication of test results
  - Inspection of hatcheries / farms / feed mills

- Regional Offices - 6
- Sub-Regional offices - 8
  - Sample collection
  - Communication of test results
  - Inspection of processing plants
MPEDA Quality Control Laboratories –

Labs involved in implementation of NRCP & PHT:

(i) QC Laboratory - Cochin (Kerala)
(ii) QC Laboratory - Bhimavaram (Andhra Pradesh)
(iii) QC Laboratory- Nellore (Andhra Pradesh).

(iv) QC Laboratory, Bhubaneswar
(This lab was being operated on management contract by a private lab. MPEDA has taken over the operations of this lab & is in the process of obtaining NABL accreditation).
National Residue Control Plan (NRCP) for Aquacultured Products:

Objectives:

- To establish a system for monitoring of the residues of Veterinary Medicinal Products (Aquaculture drugs) and Environmental contaminants.

- To establish a system of corrective actions and control in the event of residues/contaminants.

- To ensure that the aquaculture products exported from India meet the prescribed regulatory requirements of the importing countries.
NRCP:

Planning – sampling strategy, sampling level etc

NRCP schedule for Field Offices

Drawing of samples & forwarding to the Lab

Analysis of Samples

Results (online)

Repeat of Test in case of positive cases (for confirmation)

Alert Information (automatic)
In case of Non-compliant sample

Result communication by Field Office to Farmer/Processing plant

Follow-up Investigation and drawing of repeat/follow-up sample

Investigation Report & Follow-up sample to Lab & HO

Monitoring of farm/plant for 6 months / 12 months

Analysis & Results
Minimum sampling levels and frequencies:

(ref: 96/23/EC – Annexe: IV, Chapter 3.)

Group A: **one third** of the total samples:

- **all the samples** must be taken at **farm level**, at all stages of farming, including fish which is ready to be placed on the market.

Group B: **two third** of the total samples:

- the sampling should be carried out:
  - (a) preferably **at the farm**, fish ready to be placed on the market for consumption;
  - (b) either at the **processing plant**, or at **wholesale level**, on fresh fish, on condition that tracing-back to the farm of origin, in the event of positive results.

*In all cases, samples taken at farm level shall be taken from a minimum of 10 % of registered sites of production and at least 1 per 100 tonnes of annual production.*
NRCP - Substances/Residues monitored
(ref: 96/23/EC – Annexe I & II)

- GROUP-A Substances *(unauthorized substances, having anabolic effect used in Aquaculture)*:
  1. Group A1 - Stilbenes / Stilbene derivatives,
  2. A3 - Steroids
  3. A6 - (i) Nitrofuran metabolites
      (ii) Chloramphenicol
      (iii) Nitroimidazoles
*(Compounds included in Annex IV to Council Regulation (EEC) No 377/90)*
NRCP - Substances/Residues monitored (cont’d....)

- **GROUP – B:** *(VETERINARY DRUGS & ENVIRONMENTAL CONTAMINANTS)*

- **Antibacterial substances,**
  
  (Group B1) Sulphonamides, TC/OTC/CTC, Oxolinic Acid, etc).

- **Other veterinary drugs**
  
  (B2a) Anthelmintics (Ivermectin)

- **Environmental contaminants & other substances**
  
  (B3a) Organochlorine Pesticides (11 compounds) & Poly-Chlorinated Bi-phenyls (PCBs, - 6 compounds)
  
  (B3c) Chemical Elements (Hg, Cd, As, Pb)
  
  (B3d) Mycotoxins (Aflatoxins B1 & B2)
  
  (B3e) Dyes (MG & LMG)
## NRCP - Sampling Pattern - Example

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of farms enrolled with MPEDA</th>
<th>Aquaculture Production (M/T)</th>
<th>Total throughput of EU approved processing plants (RM)</th>
<th>No. of hatcheries in operation &amp; feed mills</th>
<th>No. of samples to be analysed</th>
<th>Criteria for sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Shrimp) P. monodon, P. indicus &amp; L. Vannamei</td>
<td>40177</td>
<td>327305</td>
<td>--</td>
<td>--</td>
<td>3984</td>
<td>Based on 10% of registered farms</td>
</tr>
<tr>
<td>M. rosenbergi (Scampi)</td>
<td>3546</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>36</td>
<td>1 sample per every 100 MT of production</td>
</tr>
<tr>
<td>Freshwater fishes</td>
<td>--</td>
<td>--</td>
<td>4412</td>
<td>--</td>
<td>45</td>
<td>based on throughput in approved export(EU) establishments (1:100)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4065</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Hatchery & Feed Samples

<table>
<thead>
<tr>
<th>Feed Samples</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>11</th>
<th>44</th>
<th>4 samples from each feed-mill.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery Seed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>197</td>
<td>197</td>
<td>1 sample per every registered hatchery.</td>
</tr>
</tbody>
</table>

**GRAND TOTAL** 4306
<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Total number of samples to be tested</th>
<th>Break up of samples proposed to be tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group A substances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Farms</td>
</tr>
<tr>
<td>Shrimp</td>
<td>3984</td>
<td>1328</td>
</tr>
<tr>
<td>Scampi</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>Fish</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>Sub Total</td>
<td>4065</td>
<td>1355</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Farms</td>
</tr>
<tr>
<td>Feed</td>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>Hatchery Seed</td>
<td>197</td>
<td>--</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4306</td>
<td>1377</td>
</tr>
</tbody>
</table>
**NRCP Instructions:**

1. Samples shall be collected by the designated residue monitoring officers (RMOs) only from farms enrolled under the Marine Products Export Development Authority (MPEDA).

2. Collection of sample shall be unforeseen, unexpected and effected at no fixed time and on no particular day of the week.

3. Sampling at farm level shall be - that a minimum 10% of the registered sites of production is covered in the yearly Plan, as all the registered farms in a State need to be covered over a period of time.

4. Number of samples to be collected from the processing plants under the RO/SRO shall be based on the production capacity and/or actual production of each processing plant.

5. Farms reported with residue positive cases and processing plants reported with rejections have to be closely monitored and subjected to stringent and frequent sampling.
7. More samples may be drawn from Farms situated in areas reported/suspected with presence/use of unknown chemicals/substances or indications of fraudulent activities, disease out breaks etc,.

8. Sampling levels:
   Shrimp farms: 60 - 90 days, 15 days prior to harvest.
   Scampi farms: 60-90 days, 90-120 days, 15 days prior to harvest.
   Fish farms: at any stage of production, 15 days prior to harvest.

9. Samples from the farm: details of medication within the last 4 weeks before sampling should be collected and indicated in the register as well as the slip/format accompanying the sample.

10. Processing plants- multiple samples on the same day for different parameters are allowed, provided the farms from which the Processing Plant has purchased the raw material are different.
NRCP Instructions:

11. Samples: collected in Polythene bags and properly labeled to maintain the sample integrity and traceability. The container/packing must be officially sealed prevent the substitution.

12. Signature of the farmer or his representative shall be obtained in the original sampling report. Original sampling report has to be kept with the field office and ensure that unauthorized persons cannot access the original report.

13. Register of samples: RO/SRO and RC/SRC shall maintain the register of samples collected and dispatched to the respective Laboratory.

14. RO/SRO shall note the name of the farm and identification/enrollment number in respect of each sample drawn from a processing plant, from the records of the processing plant. (96/23/EC – Articles: 11 & 12).

15. Drawing of samples:
   - from the processing plant shall be done by the residue monitoring officer of MPEDA.
   - In respect of farms - supervision of netting and actual selection of the samples shall be done by the MPEDA officer

16. Quantity (net weight) of sample drawn shall be 500 gm in case of farm/processing plant and 20 - 25 gm in case of hatchery seed.
NRCP Instructions:

17. Samples shall be forwarded to the respective MPEDA Laboratory within 3 (three) days of collection so as to reach the laboratory within 30 (thirty) hours of dispatch.

18. Samples are to be collected and delivered to the QC Laboratory concerned before 20\textsuperscript{th} of every month as per monthly target/allocation.

19. Results of the tests communicated from the respective laboratory shall be recorded in the specified columns of the register.

20. Follow up actions in case of Non-compliant (residue positive) results: The RC/SRC/RO/SRO concerned has to:

   - (i) Automatic alerts sent to Competent Authority EIC to carry out follow up action
   - (ii) Alert the processors/exporters, not to procure the raw material from the farm reported with non-compliant result (residue positive).
   - (iii) Collect follow-up sample(s) for analysis.

21. Repeat samples are to be drawn from farms / processing plants from where residue violations are reported and such farms are to be subjected to more stringent checks at least for a period of twelve months.
Pre-Harvest Test for Residues of Banned Antibiotics in Aquaculture Produce
Use of Geographic Information System (GIS) in sampling

- Enrolment of aquaculture farms in India has been carried out by the field offices of MPEDA & the National Centre for Sustainable Aquaculture (NaCSA).
- NaCSA / field offices have collected details and way points of farms by GPS.
- GIS section of MPEDA has marked the specific way points of GPS & can be verified using software like Google earth.
Objectives of PHT

➢ To establish a system for monitoring residues of banned aquaculture drugs in pre harvested aquaculture products.

➢ To ensure that the aquaculture products exported from India are free from residues of banned antibiotics.
PHT

Procedure in brief

- Request for sampling by farmer to the nearest ELISA lab
- Sample collection using GPS / GIS system for correct identification of farm
- Checking of the GPS waypoints with GIS database.
- Generating mis-match report if the waypoints do not match with database.
- An alert is generated & the data is checked by GIS section at HO & decision is taken on acceptance / rejection of sample.
- Sample acceptance at lab
- Generation of excess production alert to concerned field office & HO if farmer has requested for certification of more quantities than normal.
PHT Procedure in brief

- Based on the alert, field office conducts a farm verification & recommends / denies the excess production request.
- Sample allocation
- Sample preparation
- ELISA analysis
- Reporting of result & issue of PHTC if the sample is ELISA negative.
- If ELISA positive, sample is sent to the nearest MPEDA LC MSMS lab for confirmation.
- Based on the result from the LC MSMS lab, the sample is declared compliant/ non compliant.
• The Sample collector can locate the exact farm by using GPS.
• The farm from which sample is collected can be identified by use of GIS and GPS system.
• GIS system shows the details of enrolled farms including the details of enrolment number, farmer name, address, location of farm, species cultivated etc.
• Cast netting is commonly used for collecting samples from ponds.
• Samples collected from different corners of pond are pooled together to make a sample of 250 g.
• 250g of sample is packed in a sterile polythene plastic bag.
• Each sample is marked with a sample code/ identification number of farm
- All samples are transported to the laboratory with ice.
- Weight will be checked and the samples are coded once it reaches the laboratory
- Samples are tested for antibiotics residues.
• Under the PHT the % of positives have been showing a downward trend from (0.20%) 2013, (0.17%) 2014, (0.13%) 2015, (0.10%) 2016.

• Under NRCP the % of positives has been in the range of 7.75% (2013) to 1.31% (2015)
Suggestion

- Even though both these systems help in monitoring the presence of antibiotic residues the effectiveness is based on the action taken by the stakeholders with regards to the produce that is tested positive.
- Most of the international inspectors have recommended to try & reduce the availability of the banned antibiotics rather than testing for the presence of the banned antibiotics as this adds to cost.
- All concerned organizations & Govt. Ministries may take concerted efforts to try & reduce the availability of the banned antibiotics.