



Module for Training of Trainers (ToLTs) under National Programme for Prevention and Control of Fluorosis



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Organized by
National Centre for Disease Control, Delhi
in association with
Nutrition & IDD Cell

(Directorate General of Health Services)
Ministry of Health & Family Welfare
Government of India



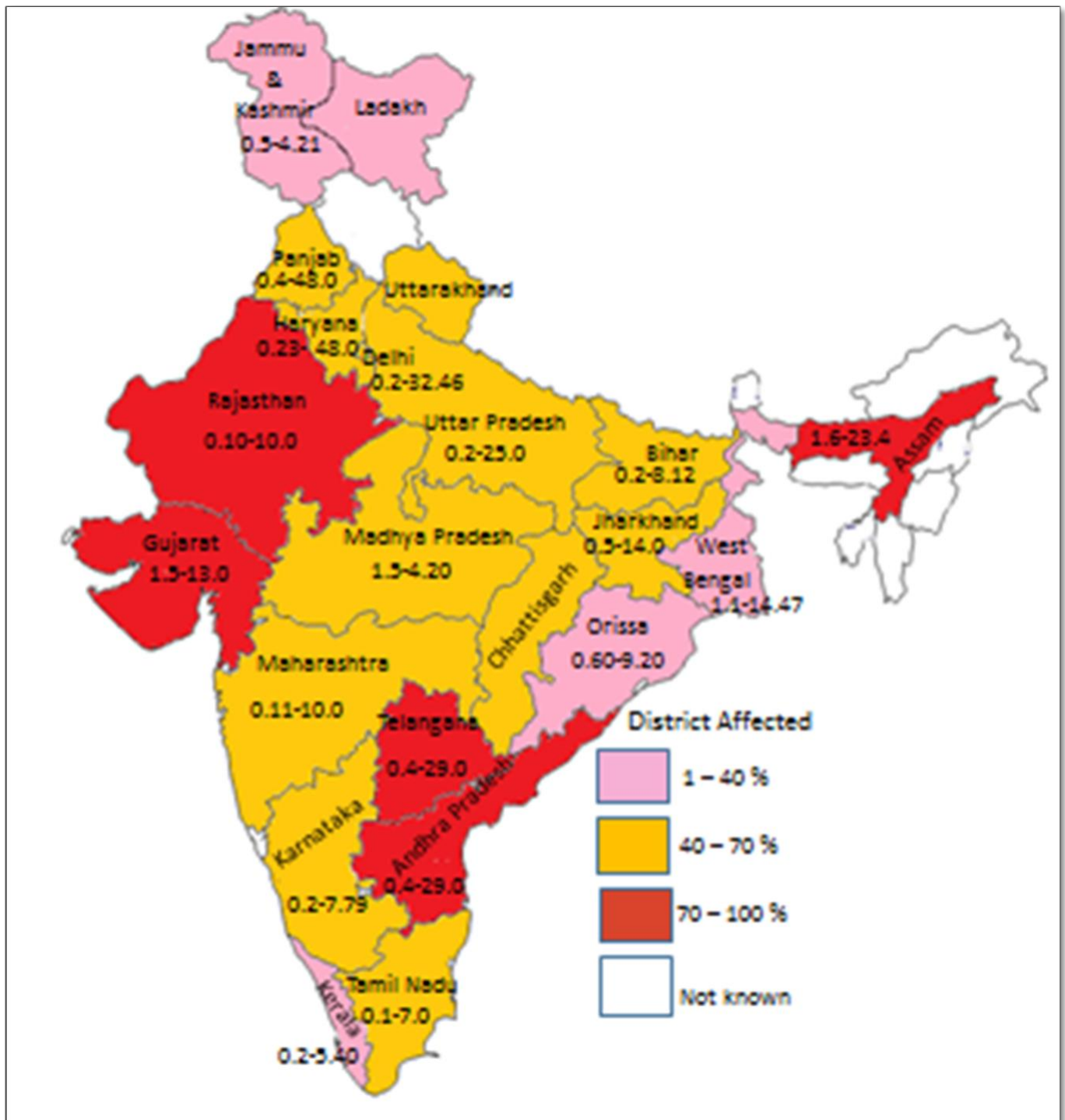
**Module for
Training of Laboratory Technicians (ToLTs)
under
National Programme for Prevention and Control of Fluorosis
(NPPCF)**

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Adapted from “Module for Training of Laboratory Technicians (ToLTs) under National Programme for Prevention and Control of Fluorosis (NPPCF), Nutrition & IDD Cell, Dte. GHS, MoHFW, January 2020.

Map of India Showing Endemicity of Fluorosis



Reference link :

<https://www.ncdc.gov.in/WriteReadData/l892s/98807285401651746944.pdf>

1. Key Objectives of Training

- To understand the problem of Fluorosis, its causes and preventive methods.
- To know the National Programme for Prevention and Control of Fluorosis in its entirety.
- To guide the community through IEC for preventing and controlling Fluorosis.
- To be in a position to implement the various components of the NPPCF Programme.

2. Introduction

Fluorosis, a crippling disease is caused by excess intake of Fluoride over a long period of time. Intake of excess Fluoride is mainly through drinking water (ground water) but food products especially black/rock salt, industrial pollutants also contribute to ingestion.

Excess Fluoride results in major health disorders like Dental Fluorosis, Skeletal Fluorosis and Non- Skeletal Fluorosis. Dental Fluorosis affects children and discolours and disfigures the teeth. Skeletal Fluorosis affects the bones and major joints of the body and in severe forms results in marked disability. Non-Skeletal Fluorosis, including Anemia, is an earlier manifestation of Fluorosis seen as gastro-intestinal complaints etc and may overlap with other diseases leading to misdiagnosis.

The Desirable limit for Fluoride in drinking water, as per BIS standards, is 1.0 mg /L (1ppm). **Permissible limit** is 1.5 mg/L (1.5ppm) but lesser the better.

To tackle the problem, Government of India launched the **National Programme for Prevention and Control of Fluorosis (NPPCF)** in 2008-09 (11th Five Year Plan) in 6 Districts to begin with subsequent expansion of the Programme in a phased manner. So far, 157 Districts of 19 States/UT have been covered. The States/ UT implementing NPPCF are Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Haryana, Jammu & Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Odisha, Punjab, Tamil Nadu, Telangana, Uttar Pradesh and West Bengal.

a) Epidemiology of Fluorosis:

Agent Factors

- Primarily it is Fluoride present in drinking water.
- When Fluoride in water is more than 1.5 mg per litre, it is toxic to health.
- pH in terms of alkalinity of water promotes the absorption of Fluoride.
- Calcium in the diet reduces the absorption of Fluoride.
- Hard water rich in Calcium reduces the Fluoride toxicity
- Fresh Fruits and Vit. C reduce the effects of Fluoride.
- Trace elements like Molybdenum enhance the effects of Fluoride.

Host Factors

- In children of school going age, seen as Dental Fluorosis.
- In third and fourth decades of life, seen as Skeletal Fluorosis.
- Males suffer more than females.
- Illiterates suffer more frequently in the Fluorotic belts.
- Where Aluminium ores are mined, it is seen as occupational health hazard.

Environmental Factors

- Fluoride-rich natural subsoil rocks due to leaching of Fluoride.
- Vegetables from high F belts.
- Fluoridated toothpaste particularly when used by children.
- Tropical climate.

b) Sources of Fluoride:

- **Natural** - Leaching of Fluoride from geological crust into drinking water.
- **Food and Beverages** - Black tea, black salt, Supari.
- **Drugs** - Some anesthetic agents, antibiotics, antidepressants, Fluoride toothpaste.
- **Pollution** - Dust from Fluoride containing soil, from mining, industrial emissions.

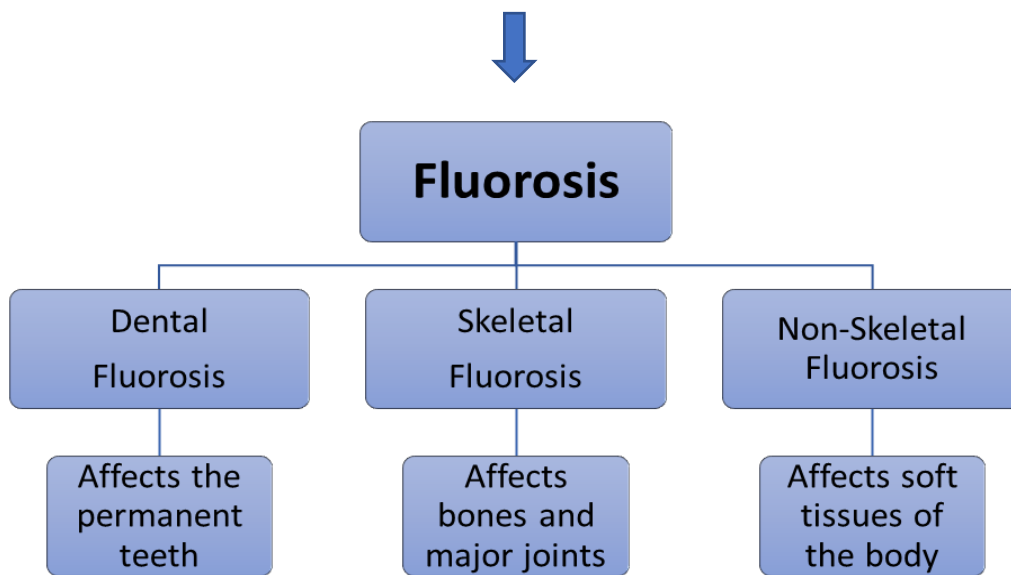
3. Fluorosis Manifestations

Pathophysiology of Fluorosis

- Fluorosis is a pathological condition caused due to excess intake of Fluoride.
- Fluoride can enter the body mainly through ingestion of fluoride available in the water, food, drugs, toothpaste etc. However, it can also enter through inhalation of Fluorine gas.

- Fluoride mostly enters the body via the gastrointestinal tract and is absorbed quickly in the stomach.
- It crosses epithelia in the form of undissociated acid Hydrogen Fluoride (HF).
- At low pH (<3.5), the more undissociated form Hydrogen Fluoride predominates, whereas at higher pH the ionized form dominates.
- In addition to crossing the stomach as undissociated acid, the majority of Fluoride absorption occurs in the small intestine and is not pH dependent.
- Carbonated hydroxyapatite found in bones reacts with HF and forms insoluble Calcium Fluoride (CaF₂).
- CaF₂ while getting cleared by the body, removes the calcium from the bone matrix.
- This process results in increased bone density (Osteosclerosis) initially, but decreased strength in bones later on and can lead to osteomalacia.

Fluorosis leads to health disorders like Dental Fluorosis, Skeletal Fluorosis and Non-Skeletal Fluorosis.



a) Dental Fluorosis

- Dental Fluorosis manifests in the form of discoloration of the teeth progressing from white, yellow, and brown to black. The discoloration may be seen as spots or streaks, invariably horizontal in orientation and always bilaterally symmetrical. The enamel may be pitted, rough and hard to clean. It becomes a permanent feature.
- Fluorosis affects both the inner and outer surface of the teeth. The commonly affected teeth are central incisors, lateral incisors and molars of the permanent dentition.

- Dental Fluorosis is irreversible and can't be cured completely. Cosmetic interventions are used.

The ingestion of toothpaste by young children (1-6 years) makes a significant contribution to their higher intake of Fluoride.

Chalky white teeth



Moderate Dental Fluorosis—Transverse yellow brown/dark brown bands



i. Symptoms of Dental Fluorosis

- Mild Dental Fluorosis is developed when Fluoride level in drinking water is between 1 and 2.5 mg/litre. In this condition, the affected teeth lose lustre and show chalkiness. In course of time, spots and transverse bands of light yellow to dark brown colour appear. Except for the disfigurement of teeth, the victims are otherwise healthy.
- Severe Dental Fluorosis is developed when Fluoride is more than 2.5 mg/litre. In this condition, the teeth become brittle and turn black owing to chipping of enamel. In course of time, there can be even loss of teeth.

ii. Treatment of Dental Fluorosis

- In many cases, Dental Fluorosis is so mild that no treatment is needed.
- Tooth whitening and other procedures to remove surface stains; bleaching the teeth may temporarily worsen the appearance of Fluorosis.
- Bonding, which coats the tooth with a hard resin that bonds to the enamel.

- Crowns.
- Veneers, which are custom made shells that cover the front of the teeth; these are used in cases of severe Fluorosis.
- Micro abrasion.

b) Skeletal Fluorosis

- Skeletal Fluorosis is a bone disease caused by excessive intake of Fluoride. Excess Fluoride intake interferes with normal bone growth, which leads to Skeletal Fluorosis. Skeletal Fluorosis can affect both the young and the adults.
- The early stage of Skeletal Fluorosis, is characterized by stiffness of the muscles, joint pain, sensations of burning, pricking, and tingling in the limbs, muscle weakness, chronic fatigue, and reduced appetite.

i. Symptoms of Skeletal Fluorosis

- Severe pain and stiffness in joints-neck, spine, hip region (pelvic girdle).
- Inability to squat.
- Ugly gait and posture.
- Increased girth, thickening and density of bone (X-ray).
- Knock knees/ Bow legs.





ii. Can Skeletal Fluorosis be cured?

Yes, in early stages, it can be prevented. In growing children, bone deformities could be reversed by Fluoride free safe drinking water and micronutrient supplementation especially calcium, vitamin D3, and Vitamin C. Once the Skeletal Fluorosis has set in adults, bone deformities cannot be corrected without surgical intervention. Crippling Skeletal Fluorosis patients require joint bone surgery and rehabilitation. The only effective remedy is prevention. This can be easily accomplished by resorting to use of safe drinking water and







improving nutritional status of the patient by incorporating Calcium, Vitamin D3, Vitamins C and Antioxidants in diet.

Fluoride in Mineralized Tissue:

- 99% of all Fluoride is present in the bone with plasma clearance of Fluoride higher than that of Calcium.
- Uptake of Fluoride is by isoionic and heteroionic exchange on the surface of bone crystallites
- Long term incorporation in the crystal lattice structure is in the form of Fluorapatite and Fluorhydroxyapatite
- Fluoride is not irreversibly incorporated in the bone.

	
<p>X Ray of Forearm showing interosseous membrane calcification</p>	<p>X Ray of Knee joint showing mature exostosis</p>

iii. Simple Tests to detect Skeletal Fluorosis in adults: -

Normal Individuals	Those individuals with joint pain
<p>Normal, healthy individual can bend his body and touch the floor/toes</p> 	<p>Unable to bend, without folding his knees</p> 
<p>Normal, healthy individual can touch the chest with chin</p> 	<p>Unable to bend the neck, touching the chest with chin not possible</p> 
<p>Normal, healthy individual can stretch the hands, fold the arms and touch the back of the head</p> 	<p>Unable to stretch the hands, fold the arms and touch the back of the head</p> 

Tests to be conducted to confirm diagnosis of Skeletal Fluorosis

- Fluoride in Drinking water source (more than 1.5 mg/litre)
- Fluoride in urine (more than 1mg/litre)
- Fluoride in serum (more than 0.5 mg/litre). Not done in the field situation
- X-ray of forearm to assess calcification of interosseous membrane

Radiological Findings

- Osteosclerosis
- Distortion of trabecular pattern
- Periosteal new bone formation or Exostoses
- Thickening of Cortex of long bone
- Calcification of interosseous membrane of forearm, ligaments, capsules, muscular attachments & tendons.
- Bowing of long bones
- Multiple growth arrest lines in the long bones
- Narrowing of Intercostal Margins

c) Non-Skeletal Fluorosis

Fluoride has multiple roles in deranging metabolic activities, besides well-defined symptoms of Dental & Skeletal Fluorosis.

- **Gastro - intestinal problems:** Gas in the stomach, loss of appetite, Consistent abdominal pain, intermittent diarrhoea/ constipation, blood in stools.
- **Anemia.**
- **Neurological manifestations:** Nervousness & depression, tingling sensation in fingers and toes,
- **Genito Urinary Problems:** excessive thirst and tendency to urinate frequently (Polydypsia and polyurea)
- **Endocrine Problems:**
 - Signs of Hypothyroidism like weight gain, lethargy, etc
 - Signs of psedo-hyperparathyroidism: New Bone formation
 - Signs of pineal gland malfunction like disturbed sleep due to altered circadian rhythm, early puberty

Central Nervous system problem: Low IQ of children

- **Muscular manifestations:** Muscle weakness & stiffness, pain in the muscle and loss of muscle power.
- Damage to G.I. Mucosa and injury to Red Blood Cells (RBCs) lead to **Anaemia / Malnutrition**, whose consequences are maternal mortality, infant mortality and low birth weight babies.
- **Elimination of Fluoride from the body leads to remarkable improvement in Non-Skeletal Fluorosis.**

Fluorosis and Pregnant women: A pregnant women should be treated as emergency in Fluorosis endemic area. She should be immediately given safe drinking water. Fluoride can cross the placental barrier, as a result if a pregnant woman continues to drink Fluoride contaminated water, the new born child may be born with Fluorosis. Moreover, post-delivery during lactation due to increase in Calcium demand, women can develop crippling Fluorosis.

4. National Programme for Prevention and Control of Fluorosis (NPPCF)

Launched in 2008-09 as a new Health Initiative during 11th Plan.

Goal: -

- To Prevent and Control Fluorosis in the country.

Objectives: -

- To collect, assess and use baseline survey data of Fluoride levels from Department of Drinking Water and Sanitation, Ministry of Jal Shakti for starting the project.
- Comprehensive management of Fluorosis cases in selected areas.
- Capacity building for prevention, diagnosis and management of Fluorosis.

NPPCF: Strategy

- Surveillance of Fluorosis in the community.
- Capacity Building at District level: -
Consultant (1), Lab. Technician (1) & Field Investigators (3) for six months.
- Establishment of diagnostic facilities through Laboratory in the affected Districts.
- Management of Fluorosis cases including treatment, surgery, reconstruction & rehabilitation.
- Health Education for prevention and control of Fluorosis.

Support is provided **to the State** for setting up of a District Fluorosis Cell with one Consultant, one Lab. Technician & 3 Field Investigators (for six months); Establishment of Labs.

NPPCF- Present Status

- NPPCF being implemented in 157 Districts in 18 States and 1 UT at present (**Annexure 1**).
- Approx.8, 415 habitations in 19 States have Fluoride levels above the permissible limit of 1.5 ppm as per IMIS data of M/o Jal Shakti, as on 1-4-2019. Approx. 60 lakh people living in these areas are at risk of Fluorosis.

Activities/ Developments under NPPCF

- Programme is under the NHM from 2014-15.
- Coordinated approach between M/o Health & F.W and Department of Drinking Water & Sanitation under M/o Jal Shakti / PHED in States and Districts for prevention of Fluorosis to provide safe drinking water to habitations affected by high Fluoride levels.
- National /Regional Review Meetings being held Annually.
- Monitoring visits are made to review implementation of NPPCF in States/ Districts
- Trainings for Trainers and Lab Technicians are held regularly
- IEC activities for creating awareness about Fluorosis and its prevention are undertaken.

Vision of NPPCF for 2030

All people in India be provided with safe drinking water, in collaboration with the Ministry of Drinking Water & Sanitation, so as to substantially reduce illnesses due to water contamination such as Fluorosis, Arsenicosis etc. and comprehensive management of Fluorosis cases is to be done.

NOTE: Please refer to the NPPCF Guidelines for the details of Survey Methodology, Training activities, Medical Management of Skeletal Fluorosis cases etc.

<http://cghealth.nic.in/ehealth/2017/Instructions/NPPCFnewguidelinebyGOI.pdf>

5. Measures for Promoting Safe Drinking Water in Fluoride Affected Areas

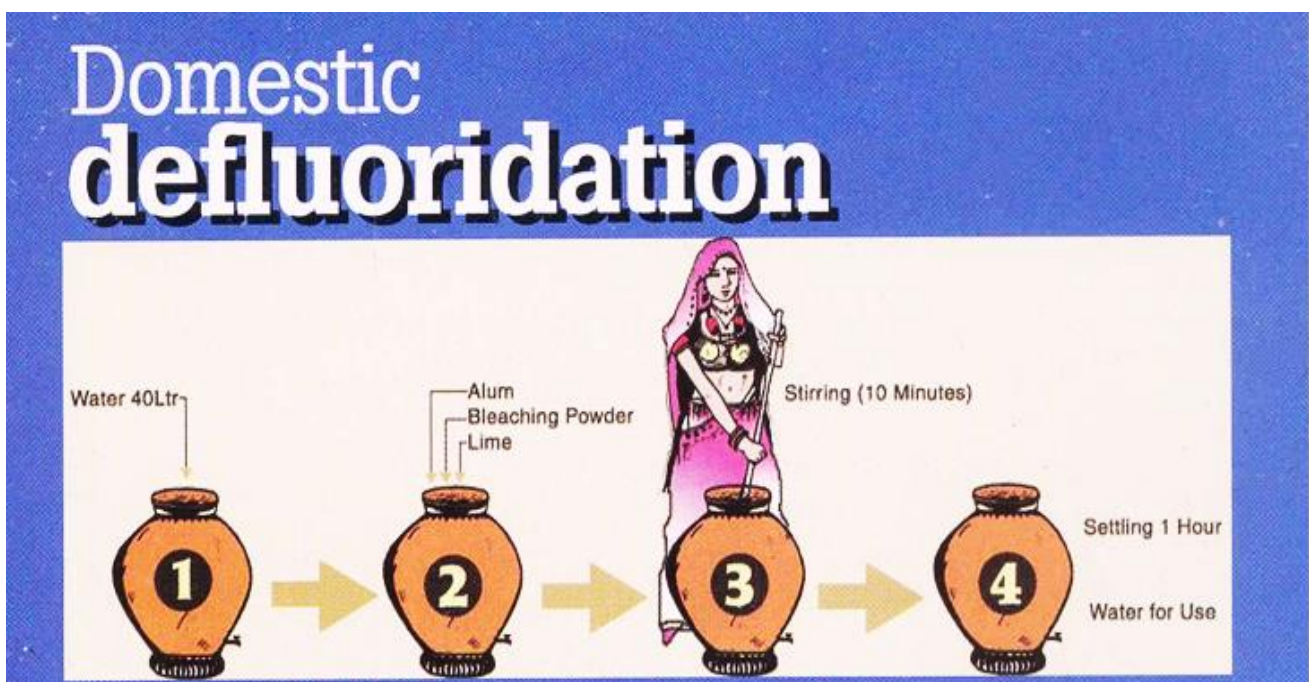
1. Use water for cooking and drinking from a safe source even if it is at a distance.
2. **Use Technologies for Fluoride Removal which can be at the individual or the community level. examples are:**
 - Electrolytic De-fluoridation- Developed and approved by NEERI. Works on the principle of electrolysis of aluminium rods. Sludge is formed, which can be dried and disposed at the disposal site. Not suitable for high TDS
 - Ion Exchange- Resin based ion exchange but is costly
 - Reverse Osmosis- Efficient in Fluoride removal and suitable for high TDS.

- Electric De-Fluoridation Plant-Aluminium Hydroxide is generated which absorbs Fluoride but this technology is not suitable where TDS is more than 700. Technology works in batches.

Domestic Defluoridation

- **Defluoridation by Nalgonda Technique (developed by NEERI, Nagpur):** This can be carried out in a container (bucket) of 60 ltr capacity with a tap 3-5 cm above the bottom of the container for the withdrawal of treated water after precipitation and setting. The raw water taken in the container is mixed slowly with adequate amount of aluminium sulphate solution (alum), lime or sodium carbonate and bleaching powder depending upon its alkalinity and Fluoride content. Alum solution is added first and mixed well with water. Lime or sodium carbonate solution then added and the water stirred slowly for 20 minutes and allowed to settle down for nearly one hour and is then withdrawn. The supernatant, which contains permissible amount of Fluoride, is withdrawn through the tap for consumption. The settled sludge is discarded.
- **Domestic defluoridation systems based on activated alumina technology (developed by IIT, Kanpur):** The technique is widely accepted because of its simplicity and high affinity of activated alumina for Fluoride. Domestic filters are also available.

The activated alumina's capacity to absorb Fluoride gets exhausted after some time, depending on Fluoride concentration in water. Trained personnel at specialised centres can regenerate this exhausted activated alumina.



6. Nutritional Interventions

Diet Editing: Withdraw Fluoride intake from all sources.

Diet Counseling: Promote consuming diet rich in all nutrients as indicated below. Intake of Vitamin C, E, calcium and anti-oxidants helps in the prevention and control of Skeletal and Non-Skeletal Fluorosis.

- **Vitamin C** - Amla, Lemon, Oranges, Tomato, Guava, Papaya, Green leafy vegetables, Sprouted cereals and pulses.
- **Vitamin E** - Vegetable oil, Nuts, Whole grain, Cereals, Green vegetables, Dried beans.
- **Calcium** - Milk, Yogurt, Sesame, Cheese, Lotus stem (Kamal Kakadi), Green leafy vegetable - Drumsticks and its leaves, Chakoda Bhaji (Cassia Tora) Zeera (Cumin seeds).
- **Anti-oxidants** - Garlic, Ginger, Carrot, White onion, Green leafy vegetables, Papaya, Pumpkin.

Food Items to be avoided: -

- Rock salt,
- Black salt.
- Tea (without milk and with lemon),
- Salted snacks smeared with black salt viz “Dalmonth”, Channa dal,
- Pickles smeared with black salt,
- Masala smeared with black salt – channa masala, jal jeera masala,
- Fruit juices (Preserved),
- Churans (Hajmola ,Hingoli, Satmola).

Dietary Counselling for Prevention of NPPCF

Please enquire about the following from households visited and provide a solution by giving them the best advice.

Question: 1 What is the source of your cooking and drinking water?

Advise: Get a sample of the water used for drinking from the Fluorosis laboratory. In case the lab is not yet established it could be tested from the PHED laboratory whether Fluoride is below 1.0 mg/L. Fluoride. More than 1.5 mg/L can cause Fluorosis.

Question: 2 Do you drink black tea (i.e., tea without milk)?

Advise: If answer is yes, then advise them not to drink black tea. It contains lot of Fluoride which is a poison. It will affect health and person who consumes it may become sick, anaemic, unwell and not able to walk properly.

Question: 3 Do you chew Supari or Tobacco?

Advise: If the answer is yes, then advise them not to chew Supari or Tobacco because it has a lot of Fluoride and shall poison you and destroy your health.

Question: 4 Do you use Black Rock Salt (Kala namak also has several names i.e. Sendha namak, Vrat ka namak, Lahori namak, etc.) in cooking, in Chaat, in Snacks, in Nimbu Pani, in Achaar or any other preparations?

Advise: If yes, advise them against its use. Rock salt contains 157 ppm Fluoride therefore pregnant women should never take Black salt. They will have low haemoglobin and baby shall have low birth weight. The brain and other organs of the child may not be properly developed.

Question: 5 Do you know there is no medicine for Fluorosis disease?

Advise: The only way to recover from Fluoride poisoning and Fluorosis is to prevent further consumption of Fluoride containing ground water and high Fluoride food items like black tea, black salt (rock salt), Supari, Tobacco etc. enhance the consumption of all nutrients on a daily basis. Drugs and tablets are not effective to cure Fluorosis. Following nutrients help to prevent Fluorosis: -

Essential nutrients. Iron + Calcium etc.

Vitamins viz. Vitamin C, Vitamin B, Vitamin A etc.

Minerals viz. Phosphorus, Sodium, Potassium, Magnesium etc.

Micronutrients viz. Iodine, Sulphur, Zinc, Chromium, Selenium etc.

Question: 6 Where does one get these nutrients from?

Advise: It is available in plenty, if one consumes fruits, vegetables and dairy products. One can consume the cheapest fruits and vegetables to get the nutrients.

- Please do **not overcook the vegetables as overcooking destroys nutrients, preferably steam** them to reduce loss of nutrients.
- Use a lot of herbs viz. Dhaniya, Pudina, Garlic, Ginger etc., make chutney and use with meals or snacks.

- Do not use too much oil in cooking, do not use oil used for frying again and again. Use less oil to fry once and use it in cooking vegetables etc, not for frying again.
- While cooking, **stir fry** by sprinkling water.
- Pressure cook the vegetables, so that nutrients are not lost.
- Try to consume green leafy vegetable every day (i.e. Palak, Methi, Sarson ka Saag or Bathua) to go with Roti or Rice.
- Try to change the fruits, each day so that different nutrients have a chance to enter the body.
- If the advice provided, is practiced regularly, one can enjoy good health.

List of Foods Recommended and Foods to be avoided may be seen at **Annexure 2.**

7. Field Visit

(i) Live demonstration of Dental Fluorosis cases

Field visit is the part of the training programme to get first-hand information on Dental and Skeletal Fluorosis. The field visit will be arranged by NCDC, Delhi to demonstrate cases of Dental Fluorosis (different grades – very mild, mild, moderate, severe).

(ii) Live demonstration of Skeletal Fluorosis cases

The cases of Skeletal Fluorosis (Genu Varum, Genu Valgum) will be identified and demonstrated in the field visit.

8. Assessment of Fluoride in The Laboratory

Importance of Fluoride analysis:

It is important to analyze Fluoride in drinking water to know the exposure to total Fluoride through drinking water which is being used for cooking also. The urinary Fluoride analysis will give the burden of Fluoride through drinking water as well as food consumed. Hence, it is important to analyze Fluoride in urine samples also.

A. Methodology for collection, labeling and preservation of Samples

(i) Sampling

- **Water:** About 30 ml water sample will be collected from the source using clean, high-density polyethylene/ polypropylene bottles with proper label indicating the date of sample collection and exact location of the sample source. Then the sample can be kept at room temperature, if not being analysed immediately.

- **Urine:** About 30 ml spot urine samples will be collected in clean, high-density polyethylene/ polypropylene bottles with screw cap, preferably with double lid, containing 2 to 4 drops of toluene as a preservative. Each sample is properly labelled with number and relevant details immediately. Then the samples can be stored at 4°C till F- analysis. Fluoride should be analyzed within a week.
- **Blood:** About 10 ml, twelve hour fasting blood sample may be collected from an individual by a trained technician in a serum vacutainer. Serum can be separated by centrifugation at 3000 rpm for 20 minute and will be stored in an ice lined refrigerator (ILR) at cold condition (-20°C) till analysis. However, blood is not analysed under NPPCF.

(ii) Fluoride Analyser (Principle and working)

The Fluoride analysis in various samples is done by Fluoride ion selective electrode method. A Fluoride ion selective electrode is a type of ion selective electrode which is sensitive to the concentration of the Fluoride ion. The Fluoride electrode consists of a single crystal made of Lanthanum Fluoride as the membrane or sensing element bonded to an epoxy body. Only Fluoride ions are mobile in the ionic conduction crystal. When the sensing element (membrane) comes in contact with a solution containing Fluoride ions, an electrode potential develops across the sensing element. This potential, which depends on the level of free Fluoride ion in solution, is measured against a constant reference (internal or external) potential with a digital Ion specific meter. The measured potential corresponds to the level of Fluoride ion in solution.

(iii) Electrode Preparation / Installation

Remove the rubber cap covering the electrode tip. To fill the filling solution, lift the spout of filling solution bottle to a vertical position. Insert the spout into the filling hole in the outer sleeve and add a small amount of filling solution to the chamber. Tip the electrode to moisten the O-ring at the top and return electrode to a vertical position. Push down on the electrode cap, allowing a few drops of filling solution to drain to wet the inner cone. Release sleeve, if sleeve does not return to its original position immediately; check to see if the O-ring is moist enough and repeat steps 2-4 until the sleeve has returned to original position. Add filling solution up to the filling hole. Electrode filling solution minimizes junction potential and Fluoride ion contamination of the sample. The filling solution level should be at least one inch above the level of sample in the beaker to ensure proper flow rate.

(iv) Limits of Detection

The limit of detection of Fluoride electrode in neutral solutions is 0.02 ppm. However, care must be taken in making determinations below 0.2 ppm. The upper limit of detection is a saturated Fluoride solution.

(v) Electrode Response

The time response of the electrode (the time required to reach 99% of the stable potential reading) depends on concentration of Fluoride in solution and it may vary from several seconds in concentrated solutions to several minutes near the limit of detection.

(vi) Maintenance of Fluoride analyzer (daily, weekly, monthly) and standardization of instrument

The solution in the electrode should not be allowed to evaporate to avoid crystallization.

- **Daily:** After analysis, close the filling solution hole with parafilm. Place the electrode in distilled water.
- **Weekly:** Close filling solution hole and store the electrode in 4M potassium chloride solution with least concentration of Fluoride.
- **Monthly:** Drain the electrode filling solution; flush the inside with distilled water and store dry with the cap to protect the sensing element.
- **Disassembly and Cleaning:** Usually, it is not recommended. However, in case of crystallization or any other problem like sample clogging cleaning can be done carefully. Drain the filling solution by pushing the cap with thumb to drain solution from the chamber. Flush the chamber with distilled water.

B. Laboratory work

- I. Preparation of Fluoride standard solutions from stock standard provided with instrument for three-point calibration.** The stock standard provided with the instrument is 100 ppm Fluoride solution. Three standards (0.1, 1.0 & 10) are required for the standardization and calibration (3 point) of the instrument.

For preparation of 0.1, 1 and 10 ppm standards for water Fluoride analysis,

- Take 10 ml of stock solution (100 ppm) in a 100 ml standard volumetric flask and make up the volume with distilled water to the mark to prepare 10 ppm standard solution.
- Take 10 ml of the 10 ppm standard solution in a 100 ml standard volumetric flask and make up the volume with distilled water to the mark to prepare 1 ppm standard solution.
- Take 10 ml of the 1ppm standard solution in a 100ml standard volumetric flask and make up the volume with distilled water to the mark to prepare 0.1 ppm standard solution.



10 ml from stock solution



10 ml from 10ppm solution



10 ml from 1ppm solution



10 ppm F solution

1 ppm F solution

0.1 ppm F solution

Preparation of 10, 1.0 and 0.1 ppm F from 100 ppm F stock solution for Water Fluoride analysis.



5 ml from stock solution



10 ml from 1ppm solution



10 ml from 0.1ppm solution



5 ppm F solution

0.5 ppm F solution

0.05 ppm F solution

Preparation of 5, 0.5 and 0.05 ppm F from 100 ppm F stock solution for Urinary Fluoride analysis

II. Standardization and Analysis of samples

Slope of ion selective electrode: Slope is defined as the change in potential (mV) observed when the concentration changes by a factor of ten and it must be in the range of 54-60 mV/decade at 25°C.

III. Calibration of Instrument: Standardization of electrode is required every 24 hour or preferably before every set of estimation, whenever type of sample changes or power failure.

Steps of calibration:

- Switch on the instrument 10 minutes before.
- Open the filling hole of electrode.
- Check the level of filling solution. It must be one inch above the level of Fluoride solution.
- Prepare 3 standards from 100 ppm Fluoride solution, which must bracket the samples.
- Rinse electrode with distilled water, blot dry and place into the lowest standard. Stir thoroughly with magnetic stirrers.
- Wait for a stable reading. Then keep second and third standards and get the slope. It must be 54-60 mV/decade. Otherwise, recalibrate the electrode.

IV. Fluoride Estimation in Water

- Switch on the instrument for stabilization.
- Allow TISAB, standards and water to come at room temperature.
- Take and label clean plastic beakers (50 ml capacity) as standard 1, standard 2, standard 3 and samples 1, 2,n
- Add 1 ml of commercially available TISAB-III to plastic beakers and add 9 ml of standards/ samples. Mix well.
- In case of TISAB II, use equal volumes of TISAB and sample / standard.
- Rinse the electrode with distilled water and blot dry.
- Keep the standards and check for the slope (it must be 54-60 my/decade).
- Keep the samples and read the value.

The participants need to practice to prepare the standard Fluoride solutions (0.1, 1.0 and 10 ppm) from Fluoride stock standard (100 ppm) provided with instrument. The standardization of Instrument is carried out with the prepared standards.

- V. Evaluation of Individual results:** If error is more than 5 %, they have to repeat the analysis of the unknown samples. The normal values for the water Fluoride is <1.0 ppm.

Preparation of 0.05, 0.5 and 5 ppm standards for Urinary Fluoride analysis

- Take 5 ml of stock solution (100 ppm) in a 100 ml standard flask and make up the volume with distilled water to the mark to prepare 5 ppm standard solution.
- Take 10 ml of the 5-ppm standard solution in a 100 ml standard flask and make up the volume with distilled water to the mark to prepare 0.5 ppm standard solution.
- Take 10 ml of the 0.5 ppm standard solution in a 100 ml standard flask and make up the volume with distilled water to the mark to prepare 0.05 ppm standard solution.

VI. Serum samples are not analysed under the NPPCF programme, however, the details are given below

- Take 10ml of stock solution (100ppm) in a 100ml standard flask and make up the volume with distilled water to the mark to prepare 10 ppm standard solution.
- Take 10 ml of the 10 ppm standard solution in a 100ml standard flask and make up the volume with distilled water to the mark to prepare 1 ppm standard solution.
- Take 10 ml of the 1 ppm standard solution in a 100ml standard flask and make up the volume with distilled water to the mark to prepare 0.1 ppm standard solution.
- Take 10 ml of the 0.1ppm standard solution in a 100ml standard flask and make up the volume with distilled water to the mark to prepare 0.01 ppm standard solution.

VII. Standardization and analysis of urine and serum samples

The 0.05, 0.5, 5 ppm standard solutions will be used for the standardization and calibration of the instrument to get the slope for urine analysis. The 0.01, 0.1, 1 ppm standard solutions will be used for the standardization and calibration of the instrument to get the slope for serum analysis. The following steps will be followed to analyse the Fluoride levels in urine and serum samples.

- Switch on the instrument for stabilization.
- Allow TISAB, standards and urine to come at room temperature.
- Take and label clean plastic beakers (50 ml capacity) as standard 1, standard 2, standard 3 and samples 1, 2, 3.....n
- Add 1 ml of commercially available TISAB-III to plastic beakers and add 9 ml of samples (urine /serum) / standards. Mix well.
- In case of TISAB II, use equal volumes of TISAB and sample / standard.

- If samples are less, urine or serum can be diluted with distilled water and the final reading should be multiplied with dilution factor.
- Rinse the electrode with distilled water and blot dry.
- Keep the standards and check for the slope (it must be 54-60 mV/decade).
- Keep the samples and read the value.

VIII. Evaluation of results of individual participants

The normal values for Urinary Fluoride is <1.5 ppm. Normal values for Serum Fluoride is <0.02ppm. Evaluation of the results of individual participants will be done and if error is more than 5%, they will repeat again with the unknown samples.

9. Linkages Between “Anemia Mukh Bharat” and Fluorosis Control

Anemia is recognised globally as well as in India as a major Public Health problem posing a continued challenge for all. Recognising the need for intensifying efforts to address all causes of Anemia using a multipronged strategy, ‘Anemia Mukh Bharat’ initiative has been designed. This is based on the technical and operational evidence from National Iron Plus Initiative (NIPI) and Weekly Iron and Folic acid (WIFS) programmes.

The target is to reduce prevalence of Anemia by 3 percentage points per year among children, adolescents and women in the reproductive age group (15–49 years), between the year 2018 and 2022.

Possible Link of Fluorosis with Anaemia

- Besides the well-defined symptoms of Dental Fluorosis, Skeletal Fluorosis and Non-Skeletal Fluorosis, excess Fluoride has other adverse effects on the body
- High Fluoride concentration in food and water leads to destruction of gastrointestinal mucosa, thus reducing the nutrient absorption, including iron and folic acid.
- Anaemia is a serious outcome of high Fluoride intake through water and food as Fluoride hampers iron absorption and hemoglobin production
- Elimination of Fluoride from the system leads to remarkable improvement

Key Interventions in Fluoride Affected Areas

- Identification of Fluoride-affected habitations

- Counselling services in the community to use safe drinking water, focus on diet corrections by dietary diversity and increased intake of Calcium, Magnesium, Vitamin C, etc.
- Periodic check-up of haemoglobin in affected habitations and appropriate treatment for the same.
- Subsequently, Iron and Folic Acid (IFA) supplementation is to be initiated.
- Capacity building by training of all public health staff, such as MO in PHC/CHC, field workers (ASHA, ANM and AWW) and others on aspects of communication and behaviour change for anemia and Fluorosis control.

Establishing Linkages of NPPCF with Anemia Mukh Bharat (AMB)

- Link and coordinate with the AMB State Nodal officer, AMB District Nodal officer, and the Block Nodal officer, for
 - Training of health functionaries and programme managers
 - IEC/BCC activities focussing on mitigation of both Anemia and Fluorosis
- Screening of pregnant women coming to antenatal clinics for urinary Fluoride. If high, counseling for elimination of Fluoride and follow up
- Identifying Research areas-support from National Centre of Excellence & Advanced Research on Anemia Control which is in Centre for Community Medicine, A.I.I.M.S, New Delhi.
- Include activities undertaken for AMB in the NPPCF Reporting mechanism

10. Information, Education and Communication (IEC) for Prevention and Control of Fluorosis

The IEC Plan covering awareness generation and to bring behavioral changes at the community level in affected districts are very important in order to prevent and control Fluorosis in the community. For this, there is a need to educate community about adverse health effects of drinking water with high level of Fluoride (above 1 ppm). To educate the community about the safe drinking water sources in their habitation and rain water harvesting measures to be adopted at the Panchayat Raj Level, are essential interventions.

Besides water contamination with Fluoride, community should be sensitized to avoid all food items with Black Rock salt-a high source of Fluoride.

The district level media team would be entrusted with the job of advocating with the various functionaries of the district including community level workers namely, ASHA, ANM, School teachers, Panchayat Members, etc. The details are tabulated below: -

National, State and District Levels:

- a) Websites of Ministry of Health & F.W./ Dte. General of Health Services (GoI)/State Govt.
- b) Press release in National/State Papers regarding the Programme,
- c) Co-ordination with Ministry of Rural Development/ Panchayati Raj Institutions/ Anganwadis to provide alternate sources of drinking water and list the sources on internet.

11. Practice and Examination by Participants for (A) Water, (B) Urinary and (C) Serum Fluoride Analysis: -

(i) Practice by participants of known and unknown samples of Water, Urine and Serum for Fluoride analysis.

The participants will practice the analysis of water, urinary and serum Fluoride levels in the unknown samples given. If the error is more than 5% they will repeat the analysis.

(ii) Practical Examination of participants for water, urine and serum Fluoride analysis.

The practical examination will be conducted for the participants to analyse the Fluoride levels in the given unknown water, urine and serum samples.

(iii) Evaluation of the results

The evaluation of the examination will be done by the Faculty of the Training Programme.

Annexure- 1

**States /UT/ Districts under National Programme for Prevention and Control of Fluorosis
(NPPCF)
(as on January,2020)**

Sl. No.	State	Total districts in the State	No. of districts under NPPCF	Districts under NPPCF
01.	Andhra Pradesh	13	9	Nellore, Guntur, Prakasam, Ananthapur, Kurnool, Krishna, Chittoor, Visakhapatnam, Srikakulam
02.	Assam	27	7	Nagaon, Kamrup, Karbi Anglong, Dhubri, Nalbari, Karimganj, Udalguri
03.	Bihar	38	11	Nawada, Banka, Aurangabad, Bhagalpur, Gaya, Jammui, Nalanda, Shekhpura, Kaimur, Munger, Rohtas
04.	Chhattisgarh	27	5	Durg, now shifted to Balod , Kanker, Kondagaon, Korba, Mahasamund
05.	Gujarat	33	4	Jamnagar, Sabarkantha, Vadodara, Banaskantha,
06.	Haryana	21	2	Mehendragarh, Mewat
07.	Jammu & Kashmir	22	1	Doda
08.	Jharkhand	24	13	Palamu, Garhwa, Chatra, Hazaribagh, Ranchi, Sahebgann, Ramgarh, Jamtara, Simdega, Dhanbad, Girih, Godda, Pakur
09.	Karnataka	30	19	Ballary, Mysore, Chikballapur, Koppal, Davangere, Tumkur, Bagalkote, Bangaluru (Urban), Bangaluru (Rural), Bijapur, Raichur, Chitradurga, Gadag, Gulbarga, Hassan, Kolar, Mandya, Ramanagara, Shimoga
10.	Kerala	14	2	Palakkad, Alapuzha
11.	Madhya Pradesh	51	15	Ujjain, Chindwada, Mandla, Dhar, Seoni, Betul, Jhabua, Raigarh, Sehore, Alirajpur, Dindori, Khargoon, Raisen, Shajapur, Ratlam
12.	Maharashtra	34	7	Nanded, Chandrapur, Latur, Washim, Yavatmal. Beed, Nagpur
13.	Odisha	30	3	Nayagarh, Angul, Nuapada
14.	Punjab	22	3	Sangrur, Ferozepur, Patiala
15.	Rajasthan	33	30	Nagaur, Ajmer, Bhilwara, Churu (Ratangarh) Dausa, Kota, Dungarpur, Rajsamand, Tonk, Bikaner, Jalore, Jaisalmer, Jodhpur, Pali, Sikar Udaipur, Swaimadhpor, Banswara, Karauli, Chittaurgarh, Ganganagar, Jhalawar, Jhunjhunu, Barmer, Alwar, Bharatpur, Sirohi, Bundi, Jaipur Pratapgarh
16.	Tamil Nadu	31	1	Dharmapuri
17.	Telangana	30	9	Mehboobnagar, Nalgonda, Karimnagar, Jagityal, Sircilla, Suryapet, Yadagiri, Wernaparth, Nagarkurnool
18.	Uttar Pradesh	75	10	Unnao, Rae Bareli, Pratapgarh, Ferozabad, Agra, Varanasi, Mathura, Sonbhadra, Ghazipur, Jhansi,
19.	West Bengal	19	6	Bankura, Purulia, Birbhum, Dakshin Dinajpur, Malda, Uttar Dinajpur
	TOTAL		157	

Dietary Advice

<p style="text-align: center;">DO NOT EAT (Food Items containing high Fluoride)</p>	<p style="text-align: center;">Recommended Foods (Proteins, carbohydrates, essential, micronutrients and antioxidants to eat on a daily basis)</p>
<p>Black tea (with or without lemon)</p>	
<p>Black rock salt in salad/raita/fruits/baked or steamed corn</p>	<p style="text-align: center;">Balanced Diet</p>
<p>Black rock salt containing food and snacks e.g “Samosa, Chole Bhature, Golgappe / Pani Poori / Bhel Poori/ Papdi Chat, Namkins/ Bhujia/ Dalmoth” etc</p>	<p>Calcium sources: Milk, Yogurt (Dahi), Paneer, Green leafy vegetables (specially Chakoda, Amaranth, Fenugreek and Drumstick leaves), Til seeds, Ragi etc.</p>
<p>“Jaljeera”/ “Nimboo pani” added with black rock salt</p>	<p>Iron sources: All Green leafy vegetables, cereals and millets especially Bajra, Ragi and pulses</p>
<p>“Achar” with black rock salt</p>	<p>Vitamin C sources: Amla, Lemon, Guava, Orange, Lime, Grapes, Tomato, Pineapple etc. Sprouted dals</p>
<p>Readymade masala packet with black salt e.g. Garam masala/chat masala/ Rajma Masala/ “Chole Masala, Pav Bhaji Masala” etc.</p>	<p>Other Antioxidants present in: All Fruits + Vegetables.</p>
<p>Hajmola/ Churan/Imli and other digestive tablets with black rock salt</p>	<p>Magnesium sources: Nuts, Cereals, Pulses, Green leafy vegetables, Lotus stem, Mango etc.</p>
<p>Canned Fruit Juices/ Fruit juice with masala salt/ preserved food items</p>	<p>Zinc sources: Pulses, Legumes, Mushrooms, Pumpkin etc.</p>
<p>Chips/Kurkure and other salty packaged snacks (Packet would reveal the ingredients)</p>	

