[1][SCHEDULE O

[*See* Rule 126]

STANDARD FOR DISINFECTANT FLUIDS

PART 1

Provision applicable to Black fluids and White Fluids.

1. *Classification.* - The disinfectants shall be classified as follows: -

(a) Black fluids (b)White fluids

(A) *Black fluids.* –

These shall be homogeneous dark brown solution of coal tar acid or similar acids derived from petroleum with or without hydrocarbon, and/or other phenolic compounds, and their derivatives and a suitable emulsifier.

(B) *White fluids.* –

These shall be finely dispersed homogeneous white to off-white emulsion consisting of coal tar acids or similar acids derived from petroleum, with or without hydrocarbons, and/or other phenolic compounds, and their derivatives.

2. *Gradation* - Each of the above classes of disinfectant fluids shall be graded on the basis of the minimum requirements in respect of:

Rideal Walker (RW) Coefficient as follows: -

Grade	Rideal Walker (RW) Coefficient (Minimum)
1.	18
2.	10
3.	5

[1]Subs.by G.O.I.Notification No. G.S.R.1243(E) dt.19.09.1979.

3. *Type.* - Each of the above grades of disinfectant fluids shall be stable in the range of temperature indicated against each type. -

Type	Stable in the range of
(I) Normal	15°C to 45°C.
(II) Winter	5 °C to 30 °C

4. *Requirements*. - All classes and grades of disinfectant fluids shall comply with the following requirements, namely: -

(1) *Stability after dilution.* - When tested by the method described hereinafter the disinfectant fluids shall be miscible with artificial hard water (for Black fluids) or with

artificial sea water (for White fluids) in all proportion from 1 per cent to 5 per cent by volume, to give emulsion which shall not break or show more than traces of separation of either top or bottom oil when kept for 6 hours at 15^{o} to 45^{o}C for Type (I) (Normal) and 5^{o}C to 30^{o}C for Type (II) (Winter).

(2) G*ermicidal Value.* - Rideal Walker Coefficient – Black fluids and White fluids shall be used tested for determination of Rideal Walker Coefficient (R.W.Coefficient) by the method described hereinafter.

(3) Storage. - Disinfectant fluids of all classes shall be stored in mild steel, tinned mild steel or other suitable containers. These shall not be stored in containers made of galvanized iron.

(4) *Labelling.* -Subject to the other provisions in these rules, the label on the container shall state-

(a) the name of the product

(ii) the name and full address of the manufacturer,

(iii) grade, type, R.W. Coefficient of product,

(iv) date of manufacture,

(v) quantity present in the container,

(vi) indications and mode of use, and

(vii) date up to which the product can be used

5. *Method of testing* -

(1) *Preparation of sample.* - The sample of disinfectant fluids to be tested should be mixed thoroughly taking care that no air is beaten into the fluid immediately before withdrawing any portion for testing. The rest portion should be withdrawn from the middle of the sample.

(2)*Method of resting stability after dilution.* -

(a) Preparation of Artificial Hard Water: 40ml of I N Hydrochloric Acid (Analytical Reagent Quality) is neutralized with a slight excess of Calcium Carbonate and filtered. The filtrate is diluted to 1000 ml with distilled water, 10 parts of this solution is further diluted to 100 parts with distilled water.

(b) Preparation *of Artificial Sea Water*: 27G of Sodium Chloride (Analytical Reagent Quality) and 5 G of Magnesium Sulphate (Analytical Reagent Quality) are dissolved in distilled water and diluted to 1000 ml. the solution is filtered before use.

(c) *Procedure*: Take 1 ml and 5 ml portions of the sample in duplicate in 100 ml stoppered measuring cylinder (IS: 878 – 1956) by means of pipettes. Dilute the sample with artificial Hard water or Artificial Sea water (as the case may be) upto 10 ml mark. Mix thoroughly by inverting the cylinders 5 times. Keep the cylinders containing the diluted fluids for 6 hours at the extremes of the temperature range specified for the particular type. The sample complies with the test if the solution shows not more than a trace of separation at its top and bottom.

(3) *Method of determination of Rideal Walker Coefficient* (R.W.C*)*

Apparatus – A loop, 4 mm in internal diameter is made at the end of 28 swg (0.376 mm) wire of platinum or platinum iridium alloy, 38mm long from the loop to the holder. The loop is bent at such an angle to the length of the wire and will facilitate in removal vertically from the surface of the liquid while keeping the place of the loop horizontal.

Incubator – Set and maintained at $37^{o}C \pm 1^{o}C$

Pipettes – Standard graduated pipettes of capacity 10 ml; 5 ml and 1 ml

Dropping Pipette – Made of delivery 0.2 ml

Medication tubes – 5 sterile plugged rimless test tubes 125 mm x 22 mm (5" x ¾") made of hard neutral glass.

Both tubes – About 2 dozens of the same description as medication tubes.

Standard measuring cylinders stopped and graduated- 500ml graduated in 10 ml-; 100ml graduated 1 ml- five. All apparatus must be scrupulously clean and sterile immediately before use.

Regents- (a) Broth- Prepare a mixture of the following ingredients:

Meat extract (Microbiological grade) 20 g Peptone (Micro biological grade) 20 g Sodium Chloride (Regent Quality) 10 g Distilled Water- 1000 ml.

Dissolve the solids in distilled water, add sufficient sodium hydroxide to neutralize the solution; then boil it to bring down phosphates and filter while hot. The broth thus prepared is then adjusted to pH 7.6 with normal Hydrochloric acid. The broth is then sterilized b autoclaving at 15 lbs pressure for 20 minutes. It is then filtered and placed in 5 ml quantities in sterilized broth tubes. The tubes of media thus prepared are sterilized by autoclaving at 15 lbs pressure for 10 minutes. The final pH of the medium should lie between 7.3 and 7.5. Further sterilization in bulk or in tubes is not possible.

(b) Test Organism- Test organism used is Salmonella typhi (NCTC 786) of which suitable culture shall be obtained from the Director, Central Drugs Laboratory, Calcutta. This

culture is maintained by weekly subculture on a nutrient agar slope (made by dissolving 2.5 per cent Agar Agar (Bacteriological grade) in broth prepared as above), incubating the subculture for 24 hours at 37°C and then storing in refrigerator at a temperature below 22°C. For the purpose of the test a little the growth from the most recent subculture in nutrient agar slope is placed in tube of R.W. broth and incubated for 23 hours at 38°C. A standard loopful is then transferred to a second tube and incubated as before. This is done at least three times before a test is carried out. Sub-cutting in broth is limited to 14 days.

(c) Standard phenol: 5 per cent W/V solution in sterile distilled water of chemically pure phenol having a crystallizing point of not less than 40.5°C is prepared. Test dilutions are prepared from this stock solution containing 1 g of phenol in each 95, 100, 105, 115 ml of the solution made. These dilutions shall be used within a week of preparation.

(d) Test dilutions of Disinfectant (sample)- The sample is prepared as described under "Preparation of samples". A test portion of 5 ml is withdrawn and discharged into about 480 ml of sterile distilled water in a 500ml glass stoppered sterile measuring cylinder and the pipette is rinsed three times or more in the clear liquid. The whole is then made up to 500ml with sterile distilled water, the cylinder is stoppered and the contents thoroughly mixed by inverting the cylinder several time. Suitable test dilutions in sterile distilled water are then immediately prepared from this stock solution.

Procedure: 5 ml of 4 chosen dilutions of the disinfectant are placed in 4 medication tubes which are than placed in a rack provided with water bath maintained at a constant temperature between 17°C and 19 °C, with the strongest dilution on the left. The fifth medication tube containing 5 ml of the particular phenol dilution is placed on the right. When the content on the medication tubes and broth culture of the test organism have reached the temperature of the water bath, starting at Zero time, 0.2 ml of the culture is added to the left hand medication tube and the tube is shaken gently. After 30 seconds the next tube is inoculated similarly and the process is repeated with each successive tube at intervals of 30 seconds until the phenol control has been inoculated. Thirty seconds after this last addition (that is 2-1/2 minutes from zero) a loopful of the well-shaken content of the tube at the extreme left is withdrawn and placed in tube containing 5 ml of the broth medium. Thirty seconds after this similar operation is performed on the second medication tube. The procedure is repeated at an interval of 30 seconds with each of the 5 medication tubes working from left to right until 4 sets of cultures have been made i.e. at 2-1/2, 5, 7-1/2 and 10 minutes respectively after exposure. In each withdrawal care should be taken to ensure that the loop is removed vertically from the surface of the liquid with its plane horizontally and without touching the side of the test tubes. The loop shall be sterilized by flaming between each operation, care being taken that the loop is cooled before being again used. The inoculated broth tubes are incubated for not less than 48 hours and not more than 72 hours at 37°C when the tubes showing growth of the test organisms will be recognized by turbidity of the broth.

Calculation of Coefficient- The R.W. Coefficient is obtained by dividing that dilution of the disinfectant which shows life of test organism in 2-1/2 and 5 minutes but no life thereafter by that dilution of the phenol which gives the same response.

A typical set of sample is given below:

Sample disinfectant Dilutions.	Time of exposures in minutes				
	2½	5	7½	10	
1: 1000	-	-	-	-	R.W.Coefficient $\frac{1200}{100} = 12$
1: 1100	+	-	-	-	
1: 1200	+	+	-	-	
1: 1300	+	+	+	-	
Phenol control					
1: 100	+	+	-	-	

(+ = growth - = No growth)

PART II

Provisions applicable to other disinfectant fluids:

Disinfectant fluids which are made with chemicals other than those specified under Part I of this Schedule shall conform to the formula or list of ingredients shown on the label.

Labelling : Subject to the provisions of rules on labeling, the label of container shall state-

(i) the name of product

(ii) the name and full address of the manufacturer;

(iii) the full formula or list of ingredients of the preparation;

(iv) date of manufacture;

(v) date up to which the product can be used;

(vi) quantity present in the container, and

(vii) indications and mode of use.

Cautionary note:

Mercury compounds shall be strictly excluded from all grades.]