Annexure 2 (b)

PESTICIDES

[Remaining: New updated list of banned pesticide (in Aug, 2108-chnages in banned list) is remaining to add in this draft because Expert person of this chapter is currently not in India so, we will get the information after she come back. Please leave one page/ Space for the new list/table of banned pesticide]

Pesticide residue

Definition. A pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs.

Table 1- Permissible limits for Pesticide Residue

Sr. No.	Substance	Limit (mg/kg)
1	Alachlor	0.02
2	Aldrin and Dieldrin (sum of)	0.05
3	Azinphos-methyl	1.0
4	Bromopropylate	3.0
5	Chlordane (sum of cis-, trans – and Oxythlordane)	0.05
6	Chlorfenvinphos	0.5
7	Chlorpyrifos	0.2
8	Chlorpyrifos-methyl	0.1
9	Cypermethrin (and isomers)	1.0
10	DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE	1.0
11	Deltamethrin	0.5
12	Diazinon	0.5
13	Dichlorvos	1.0
14	Dithiocarbamates (as CS2)	2.0
15	Endosulfan (sum of isomers and Endosulfan sulphate)	3.0
16	Endrin	0.05
17	Ethion	2.0
18	Fenitrothion	0.5
19	Fenvalerate	1.5
20	Fonofos	0.05
21	Heptachlor (sum of Heptachlor and Heptachlorepoxide)	0.05
22	Hexachlorobenzene	0.1

23	**Hexachlorocyclohexane isomers (other than γ)**	**0.3**
24	**Lindane (γ-Hexachlorocyclohexane)**	**0.6**
25	**Malathion**	**1.0**
26	**Methidathion**	**0.2**
27	**Parathion**	**0.5**
28	**Parathion-methyl**	**0.2**
29	**Permethrin**	**1.0**
30	**Phosalone**	**0.1**

31	Piperonyl butoxide	3.0
32	Pirimiphos-methyl	4.0
33	Pyrethrins (sum of)	3.0
34	Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0

Table 2- Permissible Limits for Pesticide Residue banned in India (2016)

Sr. No.	Substance	Limit (mg/kg)
1	Aldrin and Dieldrin (sum of)	0.05
2	Chlordane (sum of cis-, trans – and Oxythlordane)	0.05
3	Chlorfenvinphos	0.5
4	DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE	1.0
5	Diazinon	0.5
6	Endrin	0.05
7	Fenitrothion	0.5
8	Heptachlor (sum of Heptachlor and Heptachlorepoxide)	0.05
9	Lindane (γ-Hexachlorocyclohexane)	0.6

Note. Apart from the above, if any pesticides applied to the herb before or after harvesting should also be tested. The limit should be calculated using the following formula.

$$\frac{\times}{\times 100}$$

Where, ADI = Acceptable daily intake as published by FAO-WHO, in milligrams per kilogram of body mass,

M = body mass in kilograms (60 kg),

MDD = daily dose of herbal drug, in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{\times \quad \times}{\times 100}$$

Where, E = Extraction factor for of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

Sampling

Method. For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

Size of sampling. If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take $\sqrt{}$ + 1 samples for containers as indicated under Method, rounding up to the nearest unit if necessary.

The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in air-tight containers suitable for food contact, at a temperature below 0°, protected from light.

Reagents. All reagents and solvents are free from any contaminants, especially pesticides that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been redistilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus. Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 hours in a solution of phosphate-free detergent, rinse with large quantities of distilled water and wash with acetone and hexane or heptane.

Test for Pesticides

The following methods may be used depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry) or a different method (immunochemical methods) to confirm the results obtained. This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

Extraction (Method-I)

To 10 g of the substance being examined, add 100 ml of acetone and allow to stand for 20 min. Add 1 ml of a solution containing 1.8 µg/ml of carbophenothion in toluene. Homogenise using a high-speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml of acetone. Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40° until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of toluene. Filter through a membrane filter (45 µm), rinse the flask and the filter with toluene and dilute to 10.0 ml with the same solvent (solution A).

Purification. Examine by size-exclusion chromatography. The chromatographic procedure may be carried out using:

A stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with styrenedivinylbenzene copolymer (5 µm).

As mobile phase toluene at a flow rate of 1 ml/min.

Performance of the column. Inject 100 µl of a solution containing 0.5 g/l of methyl red and 0.5 g/l of oracet blue in toluene and proceed with the chromatography.

The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing toluene, at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

Purification of the test solution. Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat silica gel for chromatography in an oven at 150° for at least 4 hours. Allow to cool and add dropwise a quantity of water corresponding to 1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 hours using a mechanical shaker. Condition the column using 1.5 ml of hexane. Prepacked columns containing about 0.50 g of a suitable silica gel may also be used, provided they are previously validated.

Concentrate solution B in a current of helium for chromatography or oxygen-free nitrogen almost to dryness and dilute to a suitable volume with toluene (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of toluene as the mobile phase. Collect the eluate (solution C).

Extraction (Method-II)

To 25 g of the substance being examined, add 300 ml of acetonitrile: water (3:1) and homogenise using a high-speed blender for 5 min. Filter and wash the filter cake with two quantities, each of 25 ml of acetonitrile water mixture. Transfer filtrate and rinse to a separating funnel.

Add 50 ml of saturated solution of sodium chloride and mix vigorously for 30 seconds. Add 50 ml hexane to the separating funnel and extract. Repeat extraction with hexane for another two times. Collect the hexane layer and pass the combined hexane layer through sodium sulphate. Collect the hexane and evaporate to dryness. Dissolve the residue in 25 ml hexane.

Florisil column clean up. Use florisil solid phase extraction cartridges. Using bulb pipet transfer 2 ml of the hexane solution containing the pesticide residue in to the florisil cartridge. Elute with 12 ml of 15 per cent diethyl ether in hexane. Further elute with 12 ml of 50 per cent diethyl ether in hexane. Collect the elutes separately and evaporate and dry using rotary evaporator. Dissolve in 0.2 ml of n-hexane containing 10 ng/ml of carbophenothion and sonicate.

Quantitative Analysis

A. Organophosphorus Insecticides

Examine by gas chromatography, using carbophenothion as internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution. Concentrate solution B in a current of helium for chromatography almost to dryness and dilute to 100 µl with toluene.

Reference solution. Prepare at least three solutions in toluene containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve. The chromatographic procedure may be carried out using:

A fused-silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of poly (dimethyl) siloxane.

Hydrogen for chromatography as the carrier gas. Other gases such as helium for chromatography or nitrogen for chromatography may also be used provided the chromatography is suitably validated.

A phosphorus-nitrogen flame-ionisation detector or an atomic emission spectrometry detector. Maintaining the temperature of the column at 80° for 1 min, then raising it at a rate of 30°, /min to 150°, maintaining at 150° for 3 min, then raising the temperature at a rate of 4°/min to 280° and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250° and that of the detector at 275°. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 3

Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 3 Relative Retention Times of Pesticides

Sr. No.	Substance	Relative retention time
1	Dichlorvos	0.20
2	Fonofos	0.50
3	Diazinon	0.52
4	Parathion-methyl	0.59
5	Chlorpyrifos-methyl	0.60
6	Pirimiphos-methyl	0.66
7	Malathion	0.67
8	Parathion	0.69
9	Chlorpyrifos	0.70
10	Methidathion	0.78
11	Ethion	0.96

12	Carbophenothion	1.00
13	Azinphos-methyl	1.17
14	Phosalon	1.18

B. Organochlorine and Pyrethroid Insecticides

Examine by gas chromatography, using carbophenothion as the internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution. Concentrate solution C in a current of helium for chromatography or oxygen-free nitrogen almost to dryness and dilute to 500 µl with toluene.

Reference solution. Prepare at least three solutions in toluene containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using:

A fused silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of poly (dimethyl diphenyl) siloxane.

Hydrogen for chromatography as the carrier gas. Other gases such as helium for chromatography or nitrogen for chromatography may also be used, provided the chromatography is suitably validated.

An electron-capture detector.

A device allowing direct cold on-column injection. maintaining the temperature of the column at 80° for 1 min, then raising it at a rate of 30° /min to 150°, maintaining at 150° for 3 min, then raising the temperature at a rate of 4° /min to 280° and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250° and that of the detector at 275°. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 4. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 4 Relative Retention Times of Insecticides

Sr. No.	Substance	Relative retention time
1	α-Hexachlorocyclohexane	0.44
2	Hexachlorobenzene	0.45
3	β-Hexachlorocyclohexane	0.49
4	Lindane	0.49

5	δ-Hexachlorocyclohexane	0.54
6	ε-Hexachlorocyclohexane	0.56
7	Heptachlor	0.61
8	Aldrin	0.68
9	cis-Heptachlor-epoxide	0.76
10	o,p-‘DDE	0.81
11	α-Endosulfan	0.82
12	Dieldrin	0.87
13	p,p-‘DDE	0.87
14	o,p-‘DDD	0.89
15	Endrin	0.91
16	β-Endosulfan	0.92

17	**o,p-'DDT**	**0.95**
18	**Carbophenothion**	**1.00**
19	**p,p-'DDT**	**1.02**
20	**cis-Permethrin**	**1.29**
21	**trans-Permethrin**	**1.31**
22	**Cypermethrin***	**1.40**
23	**Fenvalerate***	**1.47 and 1.49**
24	**Deltamethrin**	**1.54**

***The substance shows several peaks.**

NOTE: "Government of India bans 18 pesticides after reviewing 66 out of 104 used in country recently (August, 2018)" But the detail information regarding the new banned pesticide is not available currently.