TBS/AFDC 2(231) CD2
ICS 67:250

DRAFT TANZANIA STANDARD

Specification for plastic materials for food contact applications part 3: colourants

TANZANIA BUREAU OF STANDARDS

0 Foreword

The packaging of foodstuffs is essential for their conservation, transport and handling. It also avoids contamination and maintain hygienic conditions. There is always a possibility of transfer of part of packaging materials to the packed food or foodstuffs due to intimate contact of each other. Therefore, formulation of the packaging materials must be selected to ensure that any such transfer is at a minimum and a substance which do migrate from package to the packed food cause no toxic hazards on consumption. These considerations are equally important in case of processing equipment and utensils which come in contact with foods and foodstuffs.

This standard is expected to serve as a basic guide for selection of colouring matters which are considered toxicologically safe for use in plastics and not on the manner of their actual processing or use.

In the preparation of this standard assistance has been derived from: IS 9833:2018 list of colourants for use in plastics in contacts with Foodstuffs and Pharmaceuticals published by Bureau of Indian Standards (BIS).

In reporting the result of a test or analysis made in accordance with this standard, if the final values observed or calculated, is to be rounded off it shall be done in accordance with TZS 4 *Rounding off numerical values*.

1 Scope

This standard list permitted colourants for use in plastics that may be regarded as safe for use in contact with foodstuffs.

2 Normative references

The following referenced standards referred to in the text in such a way that some or all of their content constitutes requirements of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

TZS 4: *Rounding off numerical values*.

AFDC 2(229) CD2: Determination of overall migration of constituents of plastics materials and articles intended to come in contact with foodstuffs - Method of analysis

3. Terms and definitions

For the purposes of this standard the following definitions shall apply:

3.1 colourant

ingredients that alone or in combination with other ingredients impart or alter the colour of the product.

NOTE:

1. Colourants comprise pigments (see 3.3) which are insoluble in the medium as well as dyestuffs (see 3.2) which are soluble in the medium
2. A pigment may contain the pure chemical substance and/or a surface treatment and/or additives
3. a colourant may also contains traces of impurities, which may originate from raw materials and/or the production process

4. In order to improve application properties, a colourant may contain additives.

3.2 dyestuffs

Colourants soluble in the application medium

3.3 pigments

Colourants consisting of particles, insoluble in the application medium (for example coating material or plastic)

NOTE: pigments can be further described on the basis of their chemical composition, their optical or technical properties, for example inorganic pigments, organic pigments coloured pigment, white pigment, effect pigment, corrosion inhibition-inhibiting pigment, magnetic pigment.

3.4 colourant Products

It refers to the commercially available colourant available in the market. These commercially available products contain the components responsible for the colour as well as the quantities of other substances (generally referred to as additives) designed to improve the application properties of the product., such as the dispensability colouristics, flow and flocculation resistance of pigments. Dyestuffs often contain significant amounts of diluents.

3.5 Essential colourants

The component of colourant product responsible for colour excluding additives

4. Requirements

4.1 materials

Material shall be manufactured in compliance with good manufacturing practices so that under normal or foreseeable condition of use they do not transfer their constituents to foodstuffs in quantities which could endanger human health; bring about an unacceptable change in the composition of foodstuffs or bring about deterioration in the organoleptic characteristics thereof.

4.2 when tested in accordance with AFDC 2(229) CD1, colourants used should not show visible bleeding or migration from the finished product and shall show no sign of instability or degradation during processing.

Colourants shall be sufficiently integrated within plastic material so as to preclude any visible migration in a foodstuff or under normal condition of use.

4.3 colourants used shall have a high degree of purity. It shall also comply with the requirements given in Table 1 when tested in accordance with the methods given in col 4

Table 1: Requirements for Pigment and Colourants

Sl No.	Characteristics	Requirements	Methods of test
i.	Lead, percent by mass, Max	0.01	annex G
ii.	Arsenic, percent by mass, Max	0.005	annex G
iii.	Mercury, percent by mass, Max	0.005	annex G
iv.	Cadmium, percent by mass, Max	0.010	annex G
v.	Zinc, percent by mass, Max	0.05	annex G
vi.	Selenium, percent by mass, Max	0.01	annex G
vii.	Barium, percent by mass, Max	0.01	annex G
viii.	Chromium, percent by mass, Max	0.025	annex G

ix.	Antimony, percent by mass, Max	0.025	annex G
x.	Total primary aromatic amine, Percent by mass, Max, (calculated as aniline equivalent),	0.05	Annex B
xi.	Sulphonated aromatic amine, Percent by mass, Max (calculated as aniline sulphonic acid)	0.05	Annex B
xii.	Polychlorinated biphenyl, max (mg/kg) reported as decachloro biphenyl	25	Annex C
xiii.	Carcinogenic amine listed in Annex A, max (mg/kg)	10	IS 14816

4.4 carbon black, if used, shall not be more than 2.5 percent (*m/m*). the UV absorbance of cyclohexane extract at 386 nm shall be less than 0.02 AU for a 5cm cell, it shall comply with the requirement given in table 2.

Table 2 carbon black

Sl No.	**Characteristics**	**Requirements**	**Methods of test**
i.	Toluene extract, percent by mass, *Max*	0.1	Annex D
ii.	3,4-Benzopyrene, ppm, *Max*	0.01	Annex E

5. List of Colourants for Plastics in Contacts with Foodstuffs

5.1 Organic Pigments

Table 3: List of organic pigments for Plastics in Contacts with Foodstuffs

Sl No.	*CAS No.*	*C.I. No*	*C.I. Name*
1.	2512-29-0	11680	Pigment Yellow 1
2.	6486-23-3	11710	Pigment Yellow 3
3.	5979-28-2	20040	Pigment Yellow 16
4.	6370-75-8	65405	Pigment Yellow 12
5.	12286-66-7	13940	Pigment Yellow 62
6.	5580-58-5	20038	Pigment Yellow 94
7.	5280-80-8	20034	Pigment Yellow 95
8.	5590-18-1	56280	Pigment Yellow 110
9.	29920-31-8	11738	Pigment Yellow 120
10.	79953-85-8	20037	Pigment Yellow 128
11.	30125-47-4	56300	Pigment Yellow 138
12.	36888-99-0	56298	Pigment Yellow 139
13.	71832-85-4	13960	Pigment Yellow 168
14.	96352-23-7	56160	Pigment Yellow 173
15.	77804-81-0	21290	Pigment Yellow 180
16.	74441-05-7	11777	Pigment Yellow 181
17.	67906-31-4	12830	Pigment Yellow 182
18.	65212-77-3	18792	Pigment Yellow 183
19.	129433-54-7	18795	Pigment Yellow 191
20.	3627-47-2	65410	Vat Yellow 26
21.	12236-62-3	11780	Pigment Orange 36
22.	4424-06-0	71105	Pigment Orange 43

23.	40716-47-0	11265	Pigment Orange 61
24.	72102-84-2	12760	Pigment Orange 64
25.	35869-64-8	20060	Pigment Brown 23
26.	68516-75-6		Pigment Brown 41
27.	6041-94-7	12310	Pigment Red 2
28.	2425-85-6	12120	Pigment Red 3
29.	2814-77-9	12085	Pigment Red 4
30.	6410-41-9	12490	Pigment Red 5
31.	6471-51-8	12420	Pigment Red 7
32.	6410-30-6	12335	Pigment Red 8
33.	6410-38-4	12460	Pigment Red 9
34.	6410-35-1	12440	Pigment Red 10
35.	6410-32-8	12385	Pigment Red 12
36.	3564-22-5	12350	Pigment Red 18
37.	6883-91-6	21205	Pigment Red 37
38.	6358-87-8	21120	Pigment Red 38
39.	7023-61-2	15865:2	Pigment Red 48:2
40.	15782-05-5	15865:3	Pigment Red 48:3
41.	1103-39-5	15630:2	Pigment Red 49:2
42.	17852-99-2	15860:1	Pigment Red 52:1
43.	4/9/5281	15850:1	Pigment Red 57:1
44.	6417-83-0	15880:1	Pigment Red 63:1
45.	5850-80-6	15525	Pigment Red 68
46.	72-48-0	58000:1	Pigment Red 83
47.	14295-43-3	73312	Pigment Red 88
48.	6409-74-1	60745	Pigment Red 89
49.	6535-46-2	12370	Pigment Red 112
50.	980-26-7	73915	Pigment Red 122
51.	5280-78-4	20735	Pigment Red 144
52.	5280-68-2	12485	Pigment Red 146
53.	4948-15-6	71137	Pigment Red 149
54.	56396-10-2	12290	Pigment Red 150
55.	3905-19-9	20730	Pigment Red 166
56.	2786-76-7	12475	Pigment Red 170
57.	4051-63-2	65300	Pigment Red 177
58.	5521-31-3	71130	Pigment Red 179
59.	77804-81-0	21290	Pigment Red 180
60.	2379-74-0	73360	Pigment Red 181
61.	59847-23-9	12486	Pigment Red 187
62.	3089-17-6	73907	Pigment Red 202
63.	31778-10-6	12514	Pigment Red 208
64.	1/1/33573	73905	Pigment Red 209
65.	40618-31-3	20066	Pigment Red 214
66.	68259-05-2	20055	Pigment Red 220
67.	71566-54-6	20065	Pigment Red 221
68.	52238-92-3	20067	Pigment Red 242
69.	43035-18-3	15915	Pigment Red 247
70.	84632-65-5	56110	Pigment Red 254
71.	120500-90-5	561050	Pigment Red 257
72.	70833-37-3	56270	Pigment Red 256
73.	88949-33-1	561300	Pigment Red 264

74.	1047-16-1	73900	Pigment Violet 19
75.	6358-30-1	51319	Pigment Violet 23
76.	81-33-4	71129	Pigment Violet 29
77.	5462-29-3	73385	Pigment Violet 36
78.	2379-75-1	73395	Pigment Violet 38
79.	147-14-8	74160	Pigment Blue 15:X
80.	574-93-6	74100	Pigment Blue 16
81.	1328-50-3	74140	Vat Blue 29
82.	81-77-6	69800	Pigment Blue 60
83.	482-89-3	73000	Pigment Blue 66
84.	1328-53-6	74260	Pigment Green 7
85.	1330-37-6	74255	Pigment Green 37
86.	31837-42-0	13980	Pigment Yellow 151
87.	4118-16-5	60645	Pigment Yellow 147
88.	52238-92-3	20067	Pigment Red 242
89.	250640-08-5		Pigment Orange 79
90.	84632-66-6/61951-98-2		Pigment Red 272
91.	154946-66-4	18759:1	Pigment Yellow 191:1

5.2 Dyestuffs

Table 4: List of dyestuffs for plastics in contacts with foodstuffs

SI No.	CAS No.	C.I. No	C.I. Name
1.	6370-85-0	66510	Vat Yellow 9
2.	6252-78-4	73860	Vat Red 45
3.	6492-68-8	73305	Vat Red 47
4.	482-89-3	73000	Vat Blue 1
5.	130-20-1	69825	Vat Blue 6
6.	1330-38-7	74180	Direct Blue 86
7.	128-80-3	61656	Solvent Green 3
8.	116-75-6	61568	Solvent Blue 104
9.	12236-03-2	61568	Disperse Orange 47
10.	17354-14-2	61554	Solvent Blue 35
11.	4702-90-3	48160	Solvent Yellow 93
12.	4851-50-7	625580	Solvent Green 28
13.	61969-44-6	615290	Solvent Blue 97
14.	6408-72-6	615290	Disperse Violet 26
15.	64696-98-6	48525	Solvent Brown 53
16.	6829-22-7	564150	Solvent Red 179
17.	61969-47-9/6925-69-5	564100	Solvent Orange 60
18.	75216-45-4/7576-65-0	47020	Solvent Yellow 114/Disperse Yellow 54
19.	81-39-0	68210	Solvent Red 52
20.	81-48-1	60725	Solvent Violet 13
21.	83249-52-9	56280	Disperse Yellow 241
22.	80748-21-6/54079-53-7/17772-51-9		Disperse Yellow 201
23.	20749-68-2/71902-17-5	564120	Solvent Red 135
24.	12226-78-7/81457-65-0		Solvent Blue 67

25.	6408-72-6	62025	Solvent Violet 59
26.	72968-71-9		Solvent Red 195
27.	23552-74-1/37229-23-5		Solvent Blue 45

5.3 Inorganic Pigment/Alloys

Table 5: list of inorganic pigment/alloys for plastics in contacts with foodstuffs

Sl No.	*CAS No.*	*C.I. No*	*C.I. Name*
1.	7429-90-5	77000	Aluminium
2.	7440-50-6	77400	Copper
3.	7440-22-4	77820	Silver
4.	7440-57-5	77480	Gold
5.	7440-31-5	77860	Tin
6.	7440-06-4	77795	Platinum and platinum group metal
7.	7440-50-8	77400	Bronzes of copper
8.	471-341-1	77220	Whitening (calcium carbonate)
9.	10101-41-4	77231	Calcium sulphate (gypsum, plaster of Paris)
10.	1332-58-7	77005	Kaolin
11.	13463-67-7	77891	Titan white (titanium oxide)
12.	1344-28-1	77002	Alumina
13.	637-12-7		Aluminium stearate
14.	14807-96-6 and 8005-37-6	77718	Talc
15.	51274-00-1	77492	Yellow iron oxide
16.	1345-27-3	77491	Iron oxide
17.	57455-37-5	77007	Ultramarine blue (complex silicate of aluminium and sodium sulphurated)
18.		77437	Egyptian blue (double silicate of copper and calcium)
19.	1345-16-0	77346	Cobalt blue (cobalt aluminate)
20.	1333-86-4	77266	Carbon black
21.	7727-43-7	77120	Barytes (barium sulphates)
22.	64294-91-3	77492	Sienna (natural ferric oxides)
23.	12769-96-9	77007	Pigment violet 15
24.	1308-38-9	77288	Pigment green 17
25.	1309-37-1	77491	Pigment red 101
26.	1314-13-2	77975	Pigment white 4
27.	1314-98-3	77975	Pigment white 7
28.	1317-61-9	77499	Pigment black 11
29.	57455-37-5/101357-30-6	77007	Pigment blue 29
30.	68187-51-9	77496	Pigment yellow 119
31.	7727-43-7	77120	Pigment white 21
32.	8007-18-9	77788	Pigment yellow 53
33.	12001-26-2	77019	Pigment white 20
34.	68186-90-3	77310	Pigment brown 24
35.	1345-05-7	77115	Pigment white 5

Annex A

[*Table 1, Sl No. (xiii)]*

List of Carcinogenic Amine

Sl No.	***CAS No.***	***Substance***
1.	92-67-1	4-aminobiphenyl
2.	92-87-5	Benzidine
3.	95-69-2	4-chloro-*o*-toluidine
4.	91-59-8	2-Naphthylamine
5.	97-56-3	*o*-aminoazotoluene/4-amino-2,3-dimethylazobenzene/4-*o*-Tolylazo-*o*-toluidine
6.	99-55-8	5-Nitro-*o*-toluidine
7.	106-47-8	*p*-Chloroaniline
8.	615-05-4	2,4-Diamino anisole
9.	101-77-9	4,4'-Methylenedianiline/4,4'-Diaminodiphenylmethane
10.	91-94-1	3,3'-Dichlorobiphenyl-4,4'-xylendiamine
11.	119-90-4	3,3'-Dimethoxybenzidine
12.	119-93-7	3,3'-Dimethylbenzidine/4,4'-Bi-*o*-toluidine
13.	838-88-0	3,3'-Dimethyl-4,4'-diaminodiphenylmethane
14.	120-71-8	6-Methoxy-*m*-toluidine-*p*-cresidine
15.	101-14-4	4,4'-Methylene-bis-(2-chloroaniline)/2,2'-Dichloro-4,4'-methylenedianiline
16.	101-80-4	4,4'-Oxydianiline
17.	139-65-1	4,4'-Thiodianiline
18.	95-53-4	*o*-Toluidine/2-Aminotoluene
19.	95-80-7	4-Methyl-*m*-phenylenediamine
20.	137-17-7	2,4,5-Trimethylaniline
21.	90-04-0	*o*-Anisidine-2-methoxyaniline
22.	60-09-3	4-Aminoazobenzene

Annex B

[*Table 1, SI No. (x) and (xi)*]

Determination of total primary aromatic amines

B-1 General

Analysis of primary aromatic amine (PAA) in organic colorants to ascertain quality for safe use in food contact application.

B-2 Apparatus

B-2.1 Weighing Balance, nearest to 0.0001 g.

B-2.2 Ultrasonic Bath

B-2.3 Centrifuge Capable to 3 000 rpm

B-2.4 HPLC System equipped with Gradient Elution-DAD detector and pump. UV detector can be used as optional

B-2.5 Column-250 mm x 4mm, 5µm or equivalent HPLC column

B-3 Reagents

B-3.1 hydrochloric acid solution-1N

B-3.2 sodium hydroxide solution-5N

B-3.3 HPLC Grade Methanol

B-3.4 pH strips

B-3.5 Distilled Water

B-3.6 Analytical Reference standards for amines, to test appropriate individual colourant. For example, 2,5-dichloroaniline may be used as analytical reference standards for Pigment Red 2.

B-3.7 Phosphoric acid – Analytical grade

B-4 Procedure

B-4.1 Sample Preparation

Weigh accurately 0.5g sample in 250mL capacity flask. Add 20mL methanol and 60mL 1N hydrochloric acid. Apply sonication for 5min. in ultrasonic bath at 37±2ºC and subsequently stir it for 25min at 200rpm and 37±2ºC. transfer the content to centrifuge tube and apply centrifuge for 5min at 3 000 rpm and decant clear layer in 250mL beaker through filter paper. Add 30mL, 1N hydrochloric acid in centrifuge tube and stir for 25min at 200rpm and 37±2ºC. Centrifuge this at 3000 rpm and decant clear layer in 250mL beaker through filter paper. Wash filter paper with approximately 2mL 1N hydrochloric acid and combine the filtrate with the first aqueous phase. Adjust the pH to 7 using 5N sodium hydroxide solution and transfer to 200mL volumetric flask. Make up the volume to 200mL with distilled water. Inject 10μL in HPLC directly.

B-4.2 Standards Preparation.

Weigh, to the nearest 0.1mg, 10±1 mg of each aromatic amine into a 100mL volumetric flask. Add methanol/water 8:2 (*v/v*). Place it in an ultrasonic bath for 10min to ensure complete dissolution to make standard solution for calibration curve. The stability of the mixed stock standard solution should be checked regularly. It should be stable for up to 6 months when stored in cool and dark place (27ºC). calculate the response factor (RF). Use minimum three points calibration curve.

B-4.3 instrument (HPLC) Set UP

Column: 250 mm x 4 mm, 5µm or equivalent HPLC column

Oven temperature: 40ºC

Mobile phase

A: 5mmol Diammonium hydrogen phosphate buffered to *p*H 7 with concentrated phosphoric acid

B: HPLC grade methanol

Gradient: 90 percent (*v/v*) A: 10 percent (v/v) B, in 30 min 5 percent (*v/v*) A and 95 percent (*v/v*) B

Flow rate: 0.9mL/min

Injection volume: 10*µ*L or higher depending upon limit of detection.

Injection volume: suitable to ensure the limit of detection (LOD) (5-15µL)

Run time: 45 min

Flow: 0.3mL/min

DAD mode: 240±20 nm

DAD range 200 to 800 nm

B-4.4 Calculation of response factor for each concentration

Response Factor (RF) = area of standards/concentration of standards (ppm)

B-4.5 Calculation of Individual Primary Aromatic Amine

Individual primary aromatic amine (PAA), in ppm

= (sample area x dilution factor) / (RF x Weight of sample) (g)

Recalculate to aniline equivalent in case of all amines other than those listed in Annex A

Annex C

Determination of polychlorinated biphenyl (PCB)

C-1 General

This method covers determination of total polychlorinated biphenyl (PCBs) contents in colourant material by low resolution gas chromatography coupled to high resolution mass spectrometer (LRGC-HRMS) using electron impact (EI) mode.

Application of LRGC-HRMS ensures separation/recognition of most PCB congeners are separated or recognizable at different retention times.

> Note: in case a specific PCB is to be reported which is different from any congeners directly specified by internal standard, suitable care should be taken using external window standards for identification.

C-2 Apparatus

C-2.1 Low Resolution Gas Chromatography Coupled to High Resolution Mass Spectrometer (LRGC-HRMS) using Electron Impact (EI) Mode

C-2.2 Rotary Evaporator

C-2.3 weighing balance, nearest to 0.000 1g.

C-2.4 Micropipette, with disposable pipette tips

C-2.5 Surgical Hand Gloves

C-2.6 Glass Columns

C-2.6.1 Column, 30cm long, 18mm diameter with 250mL top reservoir

C-2.6.2 Column, 30cm long, 10mm diameter with 250mL top reservoir.

C-2.7 Magnetic Stirrer and Magnetic Bar

C-2.8 Ground Joint Conical Flask-500mL

C-3 Reagents

C-3.1 concentrated sulphuric acid – analytical grade

C-3.2 n-Hexane – analytical reagent grade

C-3.3 Dichloromethane – analytical grade

C-3.4 Silica –Gel- technical grade, pore size 60 Á, 70-230 mesh, 63-200 μm

C-3.5 PCB Standard Solution (EC 4058) or Equivalent

C-3.6 *n*-Nonane

C-3.7 Celite 545 (CAS No. 68855-54-9)

C-3.8 Cesium Hydroxide

C-3.9 Silver nitrate

C-3.10 Alumina (ICN alumina B-Super I (Basic) (50-200 μm or alternative)

C-3.11 sodium sulphate, anhydrous.

C-3.12 Toluene, analytical reagent grade

C-3.13 Ethanol, analytical reagent grade

C-4 Work Conditions

Dust free environment with positive pressure inside the laboratory by hair handling unit and temperature to maintained at 22±2^{o}C

C-5 procedure

C-5.1 Preparation of Test Sample

C-5.1.1 weigh accurately 0.75-2.5 g sample into a conical flask and add 0.5-0.9 g ethanol to wet the colourant surface

C-5.1.2 Add 100 µL PCB standard (5 times diluted with n-nonane). Add 1-2g of phosphoric acid and sodium sulphate of approximately same weight as of the sample. Add 40g of 92-96 percent sulphuric acid twice, carefully with repeated shaking. Sonicate the mixture for 30-100min until homogenous dark reddish or olive colour solution is obtained.

C-5.2 Liquid –Liquid Extraction

C-5.2.1 extract sulphuric acid solution with 200mL n-hexane and rapidly stir it by ultra-sonication for at least 30 min. in case of suspected of unusually high impurity content, a mixture of 50mL n-hexane and 200mL dichloromethane to be used

C-5.2.2 Transfer the mixture to a separating funnel and collect the organic phase in a 500mL round bottom flask. Repeat the extraction steps 2 times (more if necessary) and combine all the organic phases in a round bottomed flask. In case, if possible, decant organic layer directly. In case, if oily layer found then follow the step as given as C-5.2.3. add 1-2mL n-nonane in organic phase and remove the solvent using rotary evaporator. Reduce the volume up to 1-2mL in round bottom flask, follow the clean-up procedure using 1-2mL remaining solvent

C-5.2.3 Add 50 percent aqueous potassium hydroxide solution in organic phase to make its pH 7-8, and shake out the aqueous layer twice with 100mL n-hexane, in order to control the changes of temperature due to heat of neutralization. After reaching pH of 8, transfer the solution into the separation funnel, rinse the flask with 10ml of n-hexane. Combine all the clean organic phases in a round bottom flask and dry them by adding 0.5g of anhydrous sodium sulphate. Add 1-2mL n-nonane in organic phase and remove the solvent using rotary evaporator. Reduce the volume up to 1-2mL in round bottom flask. Follow the clean-up procedure using 1-2mL remaining solvent.

C-5.3 Clean-up

C-5.3.1 Take the neat and clean glass column (size 30 cm long, 18mm diameter with 250mL top reservoir). Fill the glass column with n-hexane up to 1/3 of top reservoir. Weigh the chemical and transfer to column in following sequence. While addition of reagent shakes the column externally to avoid any air bubbles left in the column for better efficiency.

C-5.4 Alumina Column

Reagent/chemicals	***Mass (g)***
Silica gel	5
Celite: Sulphuric acid (1:1 mix)	30-33
Silica gel	5-6
Anhydrous sodium sulphate	5-7

Let n-hexane run until top layer reached. Then pre-condition with 200mL hexane: dichloromethane (4:1, v/v) and let run off to the top layer again. Discard the elute.

C-5.3.2 transfers the analyte from round bottom flask to column with help of transfer pipette and rinse the round bottom flask for 5 times with approximately 5mL hexane: dichloromethane (4:1, v/v) and transfer to multi-layer column. Add approximately 150mL n-hexane. Collet the solvent n 500mL round bottom flask. Recover the excess n-hexane by rotary evaporator until 5-7mL solution is left.

C-5.4 Alumina Column

C-5.4.1 Take the neat and clean glass column (size 30 cm long, 10 mm diameter with 250 ml top reservoir). Fill the glass column with toluene up to 1/3rd of top reservoir. Usage of n-hexane instead of toluene is also permissible. Weigh the chemicals and transfer to column in following sequence. While addition of reagent shakes the column externally to avoid any air bubbles left in the column.

Reagent/Chemicals	*Mass* (g)
Silica gel	0.3-0.5
Anhydrous alumina	12.5-13
Silica gel	0.3
Anhydrous sodium sulphate	3

C-5.4.2 After filling of the column, remove the toluene/ n-hexane up the just above the top layer of reagent.

Load concentration - Eluate of previous step into the column. Rinse the flask twice (2 times 3 mL toluene/n-hexane) and add the solvent to the column.

Pre-run - Elute off with 40mL toluene into a calibrated cylinder in case of brominated sample matrix. Elute with hexane: dichloromethane (98: 2, *v/v),* adding first 2 mL x 2 mL and finally 76mL of the eluant and collect into the same cylinder of the pre-run till reaching a volume of 120mL.

C-5.4.3 Transfer the extract solution quantitatively to a flask and evaporate to a volume of 500 µL, reduce to approximately 200µL by nitrogen blowing and transfer to a standard 1.2mL septum-sealed glass vial.

The measuring solution is obtained by rinsing the flask 2 times with 25µL n-nonane and adding the volume to the GC-vial.

C-5.5 Determination of Analytes

Refer the instrument manual for operation and analysis of PCB by gas chromatography - Mass Spectrometry (GCMS) using auto-sampler, injection volume shall be 3-10 µL.

NOTE - Perfluorotributylamine (PFTBA) tuning is performed every two months or according to instrument performance monitoring requirement

C-5.6 Calculation and Quantification Procedure

C-5.6.1 In case, if peaks are not appearing in desire windows then set the time accordingly.

Integration the M peaks in GC-quadrapole should be carried out manually in the re-quantification mode. Small peaks and especially those looking significant, but lacking even an approximate isotopic ratio as predetermined for the Analyte, response is considered
100 percent. Chemically and structurally most congers are to be ratio-calculated from the congener standard amount. Amount of each determined congener is divided by the sample weight.

C-5.6.2 *Lower Detection Limit*

C-5.6.3 *Mass Conversion Factors*

PCB below the range of I ppm cannot be measured by this method, since the internal relative standard deviation (RSO) will approach 100 percent. Report the sum of decachlorobiphenyl (DeCB) equivalent (ppm) from mono- to deca-chlorobiphenyl and apply correction factor, if any, based on the measurement of uncertainty.

Degree of Chlorination	*From CB to DeCB Equivalent*	*From DeCB to CB Equivalent*
Multiply by		
(1)	(2)	(3)
Mono-CB	2.649	0.377 50
Di-CB	2.244	0.44564
Tri-CB	1.930	0.518 13
Tetra -CB	1.706	0.586 17
Penta -CB	1.528	0.65445
Hexa-CB	1.391	0.71891
Hepta-CB	1.264	0.791 14
Octa-CB	1.158	0.86356
Nona-CB	1.073	0.93197
Oeca-CB(OeCB)	1.000	1.00000

Annex D

[Table 2, Sl No. (i)]

Determination of toluene extract

D-1 toluene discoloration method

D-1.1 General

This method covers determination of the degree discoloration of toluene by carbon black by means of spectrophotometer. This method is not applicable to high extract thermal type black.

D-1.2 Apparatus

D-1.2.1 spectrophotometer-20nm maximum spectral band pass capable of measuring transmittance in the 425±5 nm range

D-1.2.2 Absorption Cells-with an optical light path of 10 mm (light path of the toluene and not the outside for the cell) for the spectrophotometer

D-1.2.3 Analytical balance-sensitivity 0.01mg

D-1.2.4 oven-gravity convection type capable of temperature regulation of ±1ºC at 105ºC.

D-1.2.5 Filter paper – whatman No. 41 or equivalent, diameter of 150 mm.

D-1.2.6 mechanical shaker- capable of 240 strokes/min.

D-1.2.7 mortar and pestle

D-1.2.8 glass filtering funnel

D-1.2.9 beaker

D-1.2.10 Erlenmeyer flask

D-1.3 reagent

D-1.3.1 toluene

D-1.4 procedure

D-1.4.1 standardization of apparatus

Clean the absorption cell with the clean tissue. Allow the spectrophotometer to warm up at least 10 min before standardization. Rinse the cell twice with clean toluene, wipe the outside surface with clean tissue. Adjust the transmission value to 100 percent on the spectrophotometer, using the wavelength of 425 nm.

D-1.4.2 Crush pelleted samples using mortar and pestle or equivalent. Dry an adequate amount of crushed carbon black sample at 105ºC for 60min using the oven.

NOTE-an infra-red lamp must not be used for drying sample as it could vapourize some of the extractible material

D-1.4.3 allow sample to cool to room temperature in a closed container. Weigh 2.00±0.01g of sample and transfer to a 125 mL Erlenmeyer flask. Add 20 mL of toluene to the sample in the flask stopper. Without

delay, begin shaking the mixture in the mechanical shaker for 60 seconds. Immediately pour as much of the mixture as possible into the glass funnel with filter paper which has previously been prepared and inserted into a 125 mL Erlenmeyer flask. Check standardization of spectrophotometer at 425 nm. Using the same cell as used to standardize before testing, rinse the cell twice with the same filtrate to be tested. Fill the absorption cell with the filtrate and determine the percentage. Transmission on the spectrophotometer at 425 nm. If necessary, large quantities of sample and toluene may be used, keeping the ratio 10 mL of toluene per gram of carbon black unchanged, to a maximum of 5g/50mL of toluene

D-2 DETERMINATION OF THE TOLUENE EXTRACTABLE FRACTION OF CARBON BLACK

D-2.1 Principle

Carbon black is extracted in a Soxhlet apparatus with toluene. Therefore, the solvent is evaporated and the residue weighed

D-2.2 Apparatus

D-2.2.1 Soxhlet extraction apparatus

D-2.2.2 extraction vessel of paper fiber, capable of containing 10 of carbon black

D-2.2.3 laboratory glass ware

D-2.2.4 oven, adjustable up to 140±5ºC

D-2.3 solvent

D-2.3.1 toluene

D-2.4 procedure

Dry a sufficient large sample of Carbon black for one hour at 105±2ºC place 10 g of dried carbon black (weighed with an accuracy of 0.1g) in an extraction vessel previously washed with toluene. Close the vessel with cotton previously washed with toluene and place the vessel in the Soxhlet apparatus.

Extract the carbon black for 8h with 150 mL toluene, adjust the heating element of the apparatus in such a way that the extraction solvent in the extraction vessel is replenished approximately 10 times per hour. Allow to cool, remove the extraction vessel from the apparatus and concentrate the extraction to a small volume by evaporation. Wash the residue with a little toluene into a weighed glass crucible (weight: a,g) and remove the toluene by evaporation on a water bath, place the crucible in an oven for two hours at 140ºC; allow to cool in a desiccator and reweigh the crucible (weight: b,g)

D-2.5 calculation

Calculate the extractable fraction E of the carbon black using the following formula:

E= 10 x (b-a) percent

Annex E

[Table 2, SI No. (ii)]

Determination of 3,4-Benzopyrene by GC/MS

E-1 General

This method is based on gas chromatography combined with mass spectroscopy for detection of 3,4-benzopropyne at ppb level. Prior to the use of this method, appropriate sample extraction techniques must be used. In this method, a 1μL aliquot of the extract is injected into the instrument and 3,4-benzopyrene is detected by mass spectroscopy in ionization mode M/Z-50-400

E-2 Reagent

E-2.1 Dichloromethane

E-2.2 polycyclic aromatic hydrocarbon (PAH) standard-mix containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, Benz(a)anthracene, chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(gh) perylrylene, Dibenz(a) hjanthracene and indeno (1,2,3,4-cd) pyrene.

E-2.3 Internal Standards – Mix containing four isotopically labelled PAHs, namely, D12-chrysene, D10-phenanthrene, D10-acenaphthrene and D10-prylene

E-3 apparatus

E-3.1 GC/MS, equipped with auto-sampler and a 30m, 0.25ID BD-5 MS fused silica capillary column

E-3.2 Rotary Evaporator

E-3.3 helium gas

E-4 Extraction

Liquid-liquid extraction-before analysis, 0.5 μg of four internal standards: D10-phenanthrene, D10-acenaphthrene, D12-chrysene and D10-prylene are added in the sample and volume is reduced to 1mL.

E-5 Analysis

The analysis is done by GC/MS apparatus where helium gas is used as carrier gas. The column head pressure is maintained at 10 psi to give flow rate approximately 1 mL/min. injector and transfer line are maintained at 290 ºC and 250 ºC, respectively. All injection volumes are 1 μL in the split less mode. The column temperature is initially held at 70 ºC for 4 min, ranged to 300 ºC for 10 min. MS is used in the ionization mode M/Z-50-400

E-6 Preparation of Calibration Standards

Five standard solutions are prepared by diluting the standard mixtures with dichloromethane to desired concentrations. To these, add 0.5µg of four internal standards, namely, D10-acenaphthene, D10-phenanthrene, D12-chrysene, and D12-perylene. These are transferred to a capped and sealed vial.

E-7 Identification and quantification

Benzo(a) pyrene in the sample is identified by a combination of retention time match and MS match against the calibration standards quantification performed by method of internal standardization using D12-perylene.

E-8 Results

Calibration curve of benzo(a) pyrene is obtained using a series of standards solutions.

ANNEX F

TEST FOR POLYCHLOROBIPHENYLES (PCB)

F-1 Principle

The procedure comprises separation of PCB from the organochlorine pesticides residues by means of silica gel adsorption column and determination of its presence by gas-liquid chromatography (GLC).

F-2 Apparatus

M-2.1 Glass Chromatographic Column, 300 mm long, 8 mm 1D with a ground-glass socket at the upper end and a stopcock at the lower end.

F-2.2 Gas Chromatograph

Equipped with electron capture detector and coupled with printer-plotter-cum-integrator.

a) Column 1-1.8m long, 3 mm 1D; Apiezon L grease + 0.15 per cent Epikote Resin 1001 on chromosorb G (acid-washed, DMCS treated, 60-80 mesh);

b) Column 2 - 1.8 m long, 3 mm 1 D; 1.3 per cent silicone gum GE - SE-52+0.15 per cent Epikote Resin 1 001, on the same support material;

Temperature: Column oven 200°C

Detector 2000c

Injection port 200°C

F-2.3 Electron Capture Detector

F-2.4 Kuderna-Danisb Type, Evaporator, with interchangeable 10 ml graduated collection tubes. M-2.5 Snyder Columns, two-bubble micro-columns, with ground glass cones to fit the Kudema-Danish type, 10 ml collection tubes.

F-2.6 Syringe - µL capacity.

F-3 **Reagents**

F-3.1 Silica Gel, 60-100 mesh.

Heat the gel at 260°C for 4 h and, when it is cooled to 65°C, place it in a desiccator. When it has cooled finally to room temperature, weigh the required amount into a glass-stoppered flask and quickly add 2.5 ml distilled water to each 100g. Immediately stopper the flask and shake it for 1.5 h on a shaker. The silica gel is ready for use.

F-3.2 n-hexane, redistilled from potassium hydroxide pellets. When concentrated 100-fold, a 5µL portion should give no significant GCL peaks.

F-3.3 Sodium Hydroxide Solution - 5N.

F-3.4 Diethyl Ether, chromatography grade.

F-3.5 Cotton Wool, extracted with hexane and diethyl ether

F-3.6 Acetic Acid, glacial, redistilled.

F-3.7 Chromium Trioxide, re-crystallized.

F-4 Separation of PCB from the more Polar Pesticide Residues

Weigh 5.0 g of the prepared gel and rapidly transfer it, by using small amounts of hexane, to the chromatographic column, in which a plug of cotton wool has been placed just above the stopcock. The stopcock may be moistened with solvent but must not be lubricated with grease. Allow the gel to settle in the column and remove and trapped air bubbles by stirring with a glass rod. Drain the surplus hexane from the column until its meniscus just touches the surface of the gel. Introduce the cleaned-up sample extract as a solution in 1 ml of hexane, and allow the hexane to drain until the meniscus again just touches the surface of the gel. Wash the vessel that contained the extract with two I-ml portions of hexane, adding each washing separately to the column and allowing it to run just into the gel. Place a receiver, preferably a Kudema Danish evaporator, beneath the column and pass 42 ml of hexane through the column at a rate not exceeding 0.7 mL/min. Collect the eluate, stopping the elution when the meniscus reaches the top of the gel, and label this fraction I. It should contain all the PCB. Hexachlorobenzene, aldrin, pp-DDT and pp'-DDE, if present, are also eluted in this fraction. Change the receiver and pass 50 ml of a 10 percent solution of diethyl ether in hexane through the column. Collect the eluate and label it fractions 2. This contains the remainder of the organochlorine pesticide residues, including usually a small amount of pp'-DOE, and can be used for the determination of these. Concentrate fraction I to 5 ml in the Kudema-Danish evaporator and examine it by GLC with electron capture detection, on at least two stationary phases of different polarities. If further concentration is necessary, reduce the volume of the solution with a gentle stream of dry air or nitrogen at room temperature. Compare the chromatograms from fraction 1 with those given by solutions of commercial PCB preparations when injected on the same columns as well as the correspondence between peak retention times which give indication of the presence of PCB in the sample.

F-5 Oxidation of pp'-DDE in the PCB fraction

F-5.1 Adjust the volume of fraction I to 2 ml, concentrating, if necessary, as described above. Add 2 ml of acetic acid and, after replacing the micro-Snyder column, heat the tube cautiously in the steam bath until all of the hexane has evaporated, as judged by the reduction in volume. Introduce 100mg of chromium trioxide and place the tube in boiling water for 15-20 min. Cool the mixture and shake it vigorously with 2.0 mL of hexane (accurately measured) in the stoppered tube. Neutralize the acid with 6-7 mL of 5 N sodium hydroxide, solution. Shake the tube again and then set it aside until the two layers separate. Keep the tube well stoppered to prevent loss of hexane.

F-6 Detection of PCB

Inject 5 J.11 of the upper hexane layer of the oxidized mixture on to at least two GLC columns with different liquid phases, one of which should be Apiezon L. Compare the retention times of the peaks so obtained with those given by the PCB reference material. Agreement between the retention times of the peaks so obtained now indicates the presence of PCB compounds in the sample with a greater degree of certainty. Any pp'-DDE, which was present in fraction 1, will have been converted into 4, 4'- dichloro-benzophenone. Under the GLC conditions quoted, this compound has a retention time similar to that of heptachlor epoxide and so gives a peak on the chromatogram before most of the PCB isomers encountered in practice. A comparison of the traces before and after oxidation of fraction 1will show how much pp'-DOE was originally present. Adjust the volume of the hexane solution so that the individual heights ofthe PCB peaks are within the linear response range of the electron capture detector. Under the prescribed GLC conditions for the Apiezon L. column, the last of the PCB isomers normally found in wildlife tissues emerges in about 120 min after injection.

Annex G

Determination of Metallic Impurities

G-0. Both instrumental and chemical methods have been given for determination of metallic impurities. For certain metallic impurities, namely; antimony, barium, cadmium, zinc and mercury only instrumental methods have been given. For lead, arsenic, copper and chromium both instrumental and chemical methods have been given. In cases where more than one method has been given, any of these may be used. However, in case of dispute instrumental method shall be used as referee method.

G-1 Instrumental Methods

The instrumental methods described in this section may be employed in the quantitative analysis of certain metallic impurities in food additives.

Method I is applicable to substances soluble in dilute acids or mixtures of acids. Method II is used for other substances.

G-1.1 Principle

The samples are dissolved in acid or digested in a mixture of sulphuric, nitric and in some cases perchloric acids. The barium, cadmium, lead, copper, chromium, and zinc in solution are determined by conventional name atomic absorption spectroscopy. Antimony and arsenic are determined by using a hydride generation technique. Alternatively, antimony may be determined by flame atomic absorption but the hydride generation technique is more sensitive.

G-1.2 General Precautions

Because of the minute amounts of metals involved, special care shall be taken to reduce the reagent blanks to as Iowa value as possible and to avoid contamination during the test. All apparatus should be thoroughly cleaned with a mixture of hot dilute acids (I part hydrochloric acid, 1 part concentrated nitric acid, and 3 parts water) followed by thorough washing with water immediately before use.

G-1.3 Apparatus

G-1.3.1 Kjeldahl flasks - of silica or borosilicate glass fitted with an extension to the neck by means of a B24 ground joint, as shown in Fig. 4, The extension serves to condense the fumes and carries a tap funnel through which the reagents are introduced.

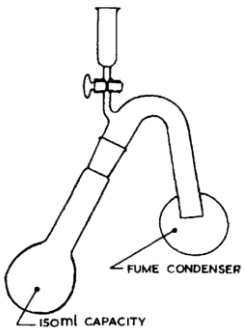

Fig. Modified Kjeldah Flask (Open Type)

G-1.3.2 Atomic absorption spectrophotometer.

Any instrument operating in the absorption mode may be used providing it has facilities for the selection of the required oxidant/fuel combination from a choice of air, argon, nitrous oxide, hydrogen and acetylene and has a wavelength range from 180 to 600 nm.

A hydride generation vessel accessory is also required and is available from all the major commercial manufacturers of atomic absorption equipment. For operations in emission mode and measurements of absorption involving the generation of a gaseous hydride, a potentiometric recorder is necessary, preferably a multi-range type covering the range 1-20 mV.

G-1.4. Reagents

13.1.4.0 Reagents shall be of an order of purity higher than accepted analytical reagent grade quality. Metal free water (see below) shall be used throughout:

a) Nitric acid - sp gr 1.42.

b) Perchloric acid - 60% (mlm) solution.

c) Sulphuric acid - 9R%.

d) Hydrochloric acid - sp gr 1.16 to 1.18.

e) Hydrochloric acid - 5 N solution prepared by dilution of reagent (d) with metal-free distilled water.

f) Water - metal-free. Distilled water may he re-distilled from an all-glass apparatus or may be passed down a column or cation exchange resin, for example, Amberlite IR 120 (H).

g) Sodium sulphate

h) Sodium borohydride pellets

i) Potassium chloride

G-1.4.1 Standards

Use commercially available standard solutions or prepare solutions as follows:

a) Standard copper solution

Dissolve 3.928g of pure copper sulphate $CuSO_4.5H_2O$ in water. dilute to 1 1000 mL at 20°C with water in a one-mark graduated flask. Dilute 10 mL to 100 mL with water in a one-mark graduated flask as required. I ml contains 100 ug Cu.

b) Standard zinc solution

Dissolve 1.00g of pure zinc powder in a mixture of 10 mL water and 5 mL hydrochloric acid [special reagent (d) (and dilute to 1 000 ml at 20°C with waler, in a one-mark graduated flask. Dilute 10 mL to 10 100 mL with water in a one mark graduated flask as required. 1 mL contains 100 µg Zn.

c) Standard chromium solution

Dilute 5.80 mL or 0.1 N potassium dichromate solution to 100 mL at 20 °C with water in a one-mark graduated flask as required. 1 mL contains 100µg Cr.

d) Standard antimony solution

Dissolve 2.668 g potassium antimony tartrate $K(SbO)C_4H_4O_6$ in distilled water, dilute to 1 000 ml at 20°C with water in a one-mark graduated flask. Dilute 10.0 ml to 100 mL with distilled water in a one-mark graduated flask as required. I ml contains 100 µg Sb.

e**) Standard lead solution**

Dissolve 1.60 g of lead nitrate, Pb $(NO_3)_2$ in nitric acid (10 mL of concentrated nitric acid diluted with 20 mL water, boiled to remove nitrous fumes, and cooled) and dilute to I 000 ml with water in a one-mark graduated flask. Dilute 10.0 mL of this solution to 500 mL at 20°C with water in a one-mark graduated flask as required. I ml contains 20 ug Pb.

f) Standard barium solution

Dissolve 1.779 g barium chloride BaCI2.2H2O in distilled water, dilute to 1000 mL at 20°C with water in a one-mark graduated flask. Dilute 10.0 mL to 100 mL with water in a one-mark graduated flask as required. 1 ml contain. 100 µg Ba,

g) Standard arsenic solution

Dissolve 1.320g of arsenous oxide, As_2O_3 by warming at a temperature not exceeding 60°C with 14mL of 5N sodium hydroxide solution in a 100mL beaker. Cool, add 0.2mL of phenolphthalein indicator and neutralize with 6N sulphuric acid. Transfer the solution to a 1000mL one-mark graduated flask containing 10 g of sodium hydrogen carbonate dissolved in water, washing out the beaker with water. Dilute to the mark with water at 20°C and mix. Dilute 5 mL of this solution to 1 000mL at 20°C with water in a one-mark graduated flask as required. 1mL contains 5µg As.

h) Standard cadmium solution

Dissolve 2.282g $CdSO4.8H_2O$ in distilled water, dilute to 1000mL at 20°C with water in a one-mark graduated flask. Dilute 10.0 mL of this solution to 500 mL at 20°C with water in a one-mark graduated flask 1 contains 20µg Cd.

G-1.5 Preparation of Test Solutions

G-1.3.0 Prepare the test solution according to Method I in the case of substances soluble in dilute acids. Use Method II for other substances.

G-1.5.1 Method I

Accurately weigh about 2.5 g of the sample and dissolve in a mixture of 4 ml of sulphuric acid and 5 ml of hydrochloric acid. Transfer the solution 10 a 50mL one-mark graduated flask. If barium is 10, he measured from the solution, add 0.095 4 g of potassium chloride. Dilute to the mark with water. Call this 'Solution A'.

G-1.5.2 Method II

Accurately weigh about 2.5g of the sample into a 100-150 mL Kjeldahl flask, and add 5mL of dilute nitric acid. As soon as any initial reaction subside heat gently until further vigorous reaction ceases and then cool. Add gradually 4mL of concentrated sulphuric acid at such a rate as not to cause excessive frothing on heating (5-11 min are usually required) and then heat until the liquid darkens appreciably in colour, that is, begins to char.

Add concentrated nitric acid slowly in small portions, heating between additions until darkening again takes place. Do not heat so strongly that charring is excessive or loss of arsenic may occur; small but not excessive amount of free nitric acid should he present throughout. Continue this treatment until the solution is only pale yellow in colour and fails to darken in colour on prolonged heating. If the solution is still coloured run in 0.5mL of the perchloric acid solution and a little concentrated nitric acid and heat for about 15 minutes, then add a further 0.5mL of the perchloric acid solution and heat for a few minutes longer. Note the total amount of concentrated nitric acid used. Allow to cool somewhat and dilute with 10mL of water. The solution should be quite colourless (if much iron is present it may be faintly yellow). Boil down gently, taking care to avoid bumping, until while fumes appear. Allow to cool, add a further 5mL of water and again boil down gently to fuming. Finally, cool, add 10mL of 5N hydrochloric acid and boil gently for a few minutes. Cool and transfer the solution to a 50 ml one-mark graduated flask washing out the Kjeldahl flask with small portions of water. Add the washings to the graduated flask and dilute to the mark with water. If barium is to be measured from the solution, add before dilution n.095 4g of potassium chloride, as an ionizing buffer to prevent ionization of barium. Call this 'Solution A'.

Prepare a reagent blank using the same quantities of reagents as used in the sample oxidation.

G-1.6 Measurement of Antimony, Barium, Cadmium, Chromium, Copper, Lead and Zinc by Atomic Absorption

15.1.6.1 Preparation of calibration curve solutions

To a series of 100mL one-mark volumetric flasks, pipette 0, 1, 2, 3, 4 and 5mL of the appropriate standard solution [standards (a) to (f) and (h) see G-1.4.11 and dilute 10 about 50mL. Add 8mL concentrated sulphuric acid [reagent (c)] and 10 mL concentrated hydrochloric acid [reagent (d)]. Shake to dissolve. In the case of barium [standard (f)], add 0.191g of potassium chloride as an ionization buffer. When solution is complete, dilute to the mark with metal free water.

These solutions then contain 1.0, 2.0, 3.0, 4.0 and 5.0µg per mL of either barium, copper, zinc, chromium or antimony respectively, or 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg per mL of cadmium or lead.

G-1.6.2 Instrumental conditions

Select the wavelength and gases to be used for the particular clement under consideration from the table below:

Element	Wavelength (nm)	Gases
Antimony	217.6	Air/acetylene
Barium	553.6	Nitrous oxide/acetylene
Cadmium	228.8	Air/acetylene
Chromium	357.9	Nitrous oxide/acetylene
Copper	324.8	Air/acetylene
Lead	283.3	Air/acetylene
Zinc	213.9	Air/acetylene

The recommended settings for the various instrumental parameters vary from model to model, and certain parameters require optimization at the time of use to obtain the best results. Instruments should therefore be adjusted as described in the manufacturer's instructions using the type of name and wavelength settings specified above.

15.1.6.3 Procedure

Set the atomic' absorption spectrophotometer to the appropriate conditions. Aspirate the strongest standard containing the clement to be determined and optimize the instrument settings to give full-scale or maximum deflection on the "hart recorder. Measure the absorbances of the other standards and plot a graph showing the net absorbance against the concentration of the element in the standard solutions. Aspirate the solution A obtained from dissolution or the wet oxidation of the sample and the corresponding blank solution and determine the net absorbance. Using the graph prepared above, determine the concentration of the clement in the sample solution;

$$Element\ in\ the\ sample, \frac{mg}{Kg} = \frac{concentration\ of\ element\ \left(\frac{g}{mL}\right)\ x\ 50}{Mass\ of\ sample\ taken\ (g)}$$

G-1.7 Measurement of Arsenic and Antimony by Atomic Absorption Hydride Technique

G-1.7.0 Arsenic and antimony are determined after preparation of their volatile hydrides which are collected either in the generation vessel itself or, in some designs, in a rubber balloon attached to the vessel. The gases are then expelled with argon into a hydrogen flame.

G-1.7.1 Preparation of calibration curve solution

Into a series of 100-mL one-mark volumetric flasks add from a burette, 0, 1,2,3,4 and 5 mL of standard arsenic or antimony solution [Standards (g) and (d)] and dilute to about 50 mL with distilled water. Add 8 ml concentrated sulphuric add [reagent (c)] and 10 ml hydrochloric acid [reagent (d)], Shake to dissolve, and when solution is complete, dilute to the mark with distilled water.

G-1.7.2 Instrumental conditions

Using the atomic absorption spectrophotometer with the appropriate hollow cathode or electrode less discharge lamp, select the wavelength for either arsenic (1937 nm) or antimony (2176 nm)

G-**1.7.3 Procedure**

Measure 5.0 ml of the strongest standard into the generation vessel, add 25mL water and 2 mL 5N hydrochloric acid [reagent (e)]. Stopper the vessel and expel any air as described in the makers instructions, filling the apparatus with argon. Isolate the vessel from the atomizer using the by-pass valve. Remove the atomizer and then quickly add 1 pellet of sodium borohydride weighing approximately 0.2 g and replace the stopper. Ensure that all the joints are secure.

When the reaction slows (20-30 sec) open the appropriate taps to allow argon to drive the generated hydride into the flame. When the hydride has all been expelled as shown by the recorder trace, return the taps to their original position and empty the vessel.

Optimize the instrument setting to give full scale deflection for the strongest standard. Measure the other standards, the sample and the blank solution using the same procedure.

Plot a graph relating peak height on the recorder to concentration of the arsenic or antimony in the standards using the net absorbance of the sample, read from the graph the concentration of arsenic or antimony in the solution.

15.1.7.4 Calculation

$$Arsenic\ or\ Antimony\ in\ the\ sample, \frac{mg}{Kg} = \frac{concentration\ of\ arsenic\ or\ antimony\ \left(\frac{g}{mL}\right)\ x\ 50}{Mass\ of\ sample\ taken\ (g)}$$

G-1.8 Determination of Mercury by atomic Absorption Cold vapour Technique

G-1.8.0 Principle

The sample is ashed by heating under reflux with sulphuric and nitric acids. The oxidation is completed by addition of potassium permanganate solution. After succession additions of hydroxylamine hydrochloride solution stannous chloride solution, the mercury chloride is measured by cold vapour atomic absorption spectrometry.

G-1.8.1 Special reagents

a) Nitric acid – sp gr 1.40

b) Sulphuric acid -sp gr 1.84

c) Sulphuric acid – approximately 3.5M. Prepare by diluting 1 volume of concentrated sulphuric acid (b), with 4 volume, of water

d) Suphuric acid – approximately 1M. prepare by diluting 1 volume of 3.5M sulphuric acid (c) with 25 volumes of water

e) Hydrochloric acid - sp gr 1.18;

f) Potassium permanganate solution - 50.0 g/L

g) Hydroxylamine hydrochloride solution -10.0g/L

h) Stannous chloride solution - Prepare by dissolving 250g of' stannous chloride ($SnCl_2.2H_2O$), in 50mL hydrochloric acid (e). Make up to 250mL with water and bubble nitrogen through the solution. Store over a few granules of metallic tin.

i) Chromic acid mixture - Dissolve 4.0g of potassium dichromate in 300mL of 3.5M sulphuric acid (c) and make up to 1 litre with water

j) Magnesium perchlorate - in granular form for gas desiccation; and

k) Mercury chloride.

G-1.8.2 Standards

Use commercially available standard solutions, or prepare the standards as follows:

a) Mercuric chloride solution 0.5mg Hg/mL. Weigh out to the nearest 0.1mg. 0.677 g of mercury chloride. Dissolve in approximately 250mL 3.5M sulphuric acid in a 1L volumetric flask, add approximately 700mL water and then potassium permanganate solution dropwise until a colouration persists. Make up to the mark with water and mix well. Renew this solution every three months.

b) Mercury chloride solution - 0.02 µg Hg/mL. Dilute the standard mercuric chloride solution 0.5 mg Hg/ml (standard (A)] by a factor of 25 000 by successive dilution with sulphuric acid [special reagent (d)], for example. 10mL made up to 250mL twice followed by 10mL made up to 400mL. Before bringing up to the mark in the final dilution, add potassium permanganate solution [special reagent (f)] dropwise until a colouration persists. Renew this solution daily.

G-1.8.3 Apparatus

All the glassware must be cleaned with hot nitric acid [special reagent (a)] and washed thoroughly with water before use.

a) Mineralization apparatus – fitted with reflux condenser (see fig. 5)

b) Bubblers – with a ground glass stopper fitted with two tubes to permit entrainment of the mercury vapour and with a calibration mark at the required volume for measurement. The capacity of the bubbler and position of the mark depend on the atomic absorption spectrophotometer used

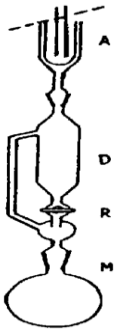

Fig: Mineralization Apparatus

Clean the bubbler successively with chromic acid mixture [special reagent (j)], tap water and double distilled water before use

(c) Water vapour absorption apparatus-containing magnesium perchlorate [special reagent (k)]

d) Atomic absorption spectrophotometer - suitable for the cold vapour determination of mercury in open or closed circuit, with recorder

G-1.8.4 Procedure

Ashing - Weigh out, to the nearest 2µg, approximately 0.5g sample containing not more than 0.5µg total mercury. Introduce the sample into the receiver flask (M), and add a few glass beads. Connect the receiver flask to the condensate reservoir (D) and close the stopcock (R)

Introduce into the reservoir 25mL of nitric acid [special reagent (a)] followed by 10mL sulphuric acid and [special regent (b)]. Mount the turn on the condenser (A) open the stopcock carefully and allow small portions of the mixture of acids to run into the receiver flask. Interrupt the flow of acids If the reaction becomes too vigorous.

Empty the reservoir into the receiver flask, mix the contents of the latter well by careful shaking and leave the stopcock open.

Heat the receiver flask carefully as soon as foaming has ceased, close the stopcock (R), continue heating and let the condensate collect in the reservoir.

Discontinue heating when the contents of the receiver flask begin to char Allow a small portion of the condensate to run to the receiver flask, close the stopcock again and resume healing the receiver flask Repeat this procedure for as long as the contents display charring when healing.

When charring has ceased, heat and add condensate as soon as white fumes appear. Continue alternately heating and adding condensate for one hour. Finally heat the contents of the flask to white fumes.

Stop heating and allow to cool to appropriately 40°C Open the stopcock and allow all the condensate to run into the receiver flask. Wash the apparatus out from the top of the condenser with 5-10 mL of water, collect the washings in the receiver flask and disconnect it from the reservoir.

Treatment of the solution - Introduce the potassium permanganate solution [special reagent (f)] dropwise into the receiver flask, with agitation, until a pink colouration persists. Note the quantity of reagent (f) used (If this quantity exceeds 10 ml, repeat the procedure 'Ashing' as above). Heat gently to boiling, then allow to cool

Pour the contents of the receiver flask into a bubbler, wash the receiver flask with water and add the washings to the contents of the bubbler.

Measure the mercury content (see below) the same day as the treatment of the solution.

Measurement of mercury content – Introduce 5mL of hydroxylamine hydrochloride [special reagent (g)] into the bubbler and make up the mark either with double distilled water or with sulphuric acid [special reagent (d)] in the case of standard solutions. Add 5 mL of stannous chloride solution [special reagent (g)], assemble the bubbler, connect it to the water vapour absorption apparatus and to the atomic absorption spectrophotometer. Set the latter in operation.

MIX the contents of the bubbler well by gently shaking, pass air or nitrogen through, measure and record. Carry out measurements as quickly as possible after the addition of stannous chloride If an open-circuit system is used, wait 30 sec before passing air or nitrogen.

Calibration curve -Introduce respectively 2, 5, 10, 15 and 25mL aliquots of the standard mercury solution [standard (b)] into bubblers and 25mL sulphuric acid [special reagent (d)] into a sixth bubbler Add potassium permanganate solution [special reagent (f)] dropwise, with agitation to each bubbler until a colouration persists.

Plot the calibration curve with the measured absorption values as ordinates and the corresponding mercury contents in microprograms as abscissae. The working standards contain 0, 0.04, 0.10, 0.20, 0.30 and 0.50µg of mercury, respectively.

Method of addition – the method of addition may be used if an open circuit system is used

Place one of the working standard solutions in a bubble and add an aliquot portion of the sample solution obtained after treatment. The quantity of mercury in the bubbler must lie in the range in which the photometer gives a linear response. Measure the mercury content as described above. If necessary, carry out several such determinations, using different working standard solutions.

Blank determination - Carry out all the operations, from ashing to measurement, except for introduction of the sample. When treating the solution, add a quantity of potassium permanganate solution [special reagent (f)] equal to that used for the experimental sample.

G-1.8.5 Calculation

Read off from the calibration curve the quantities, in µg, of mercury corresponding to the measured absorption values.

Subtract the quantity of mercury found in the blank from that found in the sample:

$$Hg\ in\ the\ sample, \frac{mg}{Kg} = \frac{Net\ mass\ of\ mercury\ \left(\frac{\mu g}{mL}\right)}{Mass\ of\ sample\ taken\ (g)}$$

G-2 Chemical Methods

G-2.0 Chemical methods have been given for determination of lead (Pb), arsenic (As), Copper (Cu) and chromium (Cr).

15,2.1 Test for Lead

15.2.1.1 Apparatus

a) Digestion Funnel

b) Separatory Funnel

15.2.1.2 Reagents

a) Nitric acid - 65 percent.

b) Sulphuric acid - sp gr 1.84.

e) Ammonium acetate-citrate solution - Dissolve 12.5g of ammonium acetate and 12.5g of ammonium citrate in water, add concentrated ammonia until the solution is alkaline to thymol blue paper and add water to 100mL. Purify with 0.002 percent m/v solution of dithizone in carbon tetrachloride, and finally shake the solution with carbon tetrachloride to remove excess of dithizone.

d) Ammonia solution - 25 percent.

e) Carbon tetrachloride

f) Ammonium hydroxide - 0.2 N.

g) Potassium cyanide - 10 percent.

h) Hydroxylamine hydrochloride solution - 10 percent.

j) Dithizone solution - 0.1 percent (m/v), purified by the following procedure:

Dissolve the dithizone in chloroform and treat it with ammonia. Add mineral acid. Precipitate shall be pure dithizone. The aqueous ammoniacal solution obtained from chloroform solution of dithizone should be colourless; otherwise, further purification as mentioned above should be carried out.

k) Buffer pH2 - Add 11.90 mL of 0.2 M hydrochloric acid and 88.10 mL of 0.2M potassium chloride in a 200-mL volumetric flask and add water to volume.

G-2.1.3 Procedure

The limit test described for lead is designed to show if a sample contains more than 10 mg/kg or 20 mg/ kg of lead. The sample is digested with nitric and sulphuric acids, and a clear solution of the digest is prepared.

Digestion - Weigh 1.0 g of sample for substances with 10 mg/kg limit or 0.5 g for those with 20 mg/ kg limit. Place with 5mL of water, 5mL of 65 percent nitric acid and 5 mL of sulphuric acid in a digestion flask. Warm slightly. If foaming becomes excessive, add a little water. Evaporate the mixture. Maintain strongly oxidizing conditions in the flask during digestion by adding cautiously small quantities of nitric acid whenever the mixture begins to turn brown or dark. Continue digestion until organic matter is destroyed and sulphuric trioxide fumes are copiously evolved. The final solution should be colourless or at most slight straw colour.

To remove nitrosyl-sulphuric acid, after partial cooling transfer the residue to a dish, rime with 25mL of water, evaporate and heat again to fuming point. Add 25mL of water and evaporate and heat again. Solution of digest - Allow to cool, add 20mL of ammonium acetate-citrate solution, and allow again to cool, neutralize to about pH 7 with 25 percent ammonia and boil if necessary, to dissolve calcium sulphate. Cool the clear solution. Shake the solution with 10mL of carbon tetrachloride and discard the lower layer.

Test with dithizone - To a 100mL separatory funnel, add 10mL of 0.2N ammonium hydroxide, 2mL of 10 percent potassium cyanide, 2mL of 10 percent hydroxylamine hydrochloride solution and 2 mL of 20 mg per litre solution of dithizone in carbon tetrachloride. Shake the separatory funnel for a few minutes and discard the carbon tetrachloride layer. Add 2mL of carbon tetrachloride, shake and discard again the carbon tetrachloride layer. Add the solution of digest to the separatory funnel. Again, add 5mL of dithizone solution in carbon tetrachloride and shake vigorously for a few minutes. The carbon tetrachloride layer becomes red according to the amount of lead present.

Treat simultaneously a standard solution containing 10µg of lead in the same manner as the solution of the digest. Evaluate the quantity of lead in the digest by comparing the colour of the carbon tetrachloride layers.

Ensure that the red colour is due to lead by shaking the carbon tetrachloride layer obtained from the digest solution with 10 mL of a buffer pH2. The red colour will turn green if it is due to lead.

G-2.2 Test for Arsenic

G-2.2.0 Arsenic may be tested by either the method given below for routine purposes.

G-2.2.1 Apparatus

a) Distillation apparatus - as shown in Fig. 6

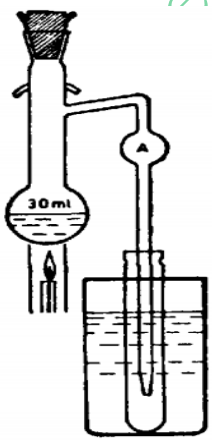

Fig. 6

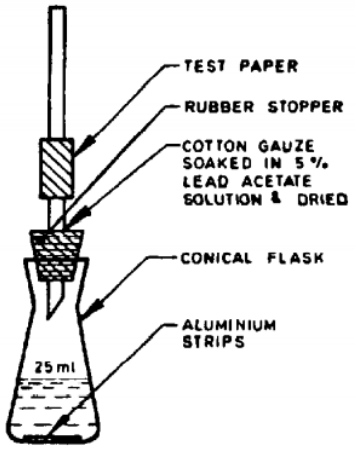

Fig. 7

b) Conical flask - 25 mL close. as shown in Fig. 7

G-2.2.2 Reagents

a) Sulphuric acid - sp gr 1.84.

b) Potassium permanganate solution - 0.1N.

c) Ferrous sulphate - freshly powdered.

d) Hydrochloric acid - 38 percent.

e) Potassium bromide solution - 20 percent.

f) Aluminium strips - 8 mm x 8 mm x I mm.

g) Tin chloride solution - 2 percent tin chloride in 10 percent hydrochloric acid.

h) Test paper - Soak strips of filter paper in saturated ethanolic solution of mercuric bromide and allow to dry.

G-2.2.3 Procedure

The limit test prescribed for arsenic shows whether a sample contains more than 3mg/kg of arsenic.

Digestion of 1 g sample and removal of nitrosyl-sulphuric acid as described in para 3 of G-2.1.3. Allow the digest. with 5 mL sulphuric acid. to cool.

Distillation (according to Snyder) - Pour the digest into the distillation flask (Fig. 6). Rinse the dish twice using each time 2.5mL of water and add the water to the digest. Cool the solution. Add some drops of potassium permanganate solution until the red colour persists. Add 0.250g of freshly powdered ferrous sulphate, 2.5mL of 38 percent hydrochloric acid and 0.1mL of potassium bromide solution.

Close the flask, place a reagent tube containing 8 mL of water in a beaker with water as shown in Fig. 6.

Heat with micro-burner. In the beginning a slow stream of air bubbles appear, followed after 3 to 5 minutes by hydrochloric acid gas. Heat then a little stronger so that the solution boils. After bulb A has become very hot, boil for 40 seconds. There upon lower the reagent tube and remove the flame. The hydrochloric acid content of the distillate shall be 9 to 10 percent only then has arsenic been completely distilled over; moreover, this acid concentration is required for the test given below;

Test with mercury bromide paper (Mayercon – Bergerel) - The distillate should be transferred to a conical flask of 25 ml capacity. The flask should be dosed with the device as shown in Fig. 7 containing a small disk of test paper. Add 3 pieces of aluminium strips, 1 mL of tin chloride solution and Immediately close the flask with the stopper. Allow the flask to stand in a water-bath of 25° to 30°C for 50 for 60 minutes. At the same time carry out a parallel test using a solution of 2, 4, 6 or 8µg of arsenic in 10 mL of 10 percent hydrochloric acid in place of the test sample. Compare the colour of the test paper for evaluating arsenic content of the sample.

G-2.3 Determination of Copper

G-2.3.1 Reagents

a) Citric acid - solid.

b) Ammonium hydroxide solution - sp gr 0.92 (not less than 27 percent ammonia).

c) Concentrated hydrochloric acid

d) Dithizone (diphenyl thiocarbazone] solution - 0.1 percent (m/v) in chloroform.

e) Concentrated nitric acid

f) Concentrated sulphuric acid

g) Citric acid solution - 5 percent (m/v). aqueous.

h) Gum arahic solution - one percent.

j) Sodium diethyl dithiocarbamate solution - 0.2 percent (mlv). aqueous, freshly prepared.

k) Standard strong soluiion of copper - Dissolve 0.392 5g of pure crystallized copper sulphate ($CuSO_4$. $5H_2O$) in water and make up the volume to 100 mL, this solution contains one milligram of copper per milliliter.

m) Standard dilute solution of copper - Dilute one millilitre of the standard strong solution of copper to 100 mL in a graduated flask, one millilitre of this solution contains 0.01 µg of copper.

G-2.3.2 Procedure

Take a suitable aliquot of the test solution prepared as described above and add 28 of citric acid. Neutralize exactly with the ammonium hydroxide solution using a piece of litmus paper and acidify with one millilitre of concentrated hydrochloric acid. Cool and transfer to a separating funnel. The total volume of the solution should be about 100mL.

Extract the copper by shaking with three successive portions of 5mL of the solution of dithizone, shaking thoroughly for a minute for each extraction. Separate the dithizone layers and wash the combined dithizone extracts with about 10 ml of water. Transfer the dithizone extract to a tube of heat-resistant glass and evaporate the chloroform on a water-bath.

Heat the copper-dithizone residue in the test tube with one millilitre or concentrated sulphuric acid and a little of nitric acid until all organic matter is destroyed. Add 5mL of water and re-heat to fuming stage. Cool, dilute with water and transfer the whole of the solution or a measured volume of the solution, depending upon the amount of copper present, to a Nessler cylinder. Add one millilitre of the citric acid solution and 4 mL of the ammonium hydroxide solution followed by 5 mL of the gum arabic solution and make up the volume to 50 mL with water. Add 5mL of the sodium diethyl dithiocarbarmate solution and match the colour by adding the standard dilute solution of copper [reagent (m)] to a control cylinder containing the same quantities of reagents as present in

the test solution. Calculate the copper content of the material in parts per million from the known volume of the standard dilute solution of copper required for matching.

G-2.4 Determination of Chromium

G-2.4.1 Reagents

a) Magnesium nitrate solution - 25 percent (m/v).

b) Strong sulphuric acid solution - 4 N.

c) Potassium permanganate solution - 0.1 N.

d) Sodium azide solution - 5 percent (mlv).

e) Sodium dihydrophosphate - 4 M.

f) Diphenyl-carbazide solution - Dissolve 125mg of diphenyl-carbazide (C_6H_5.NH. NH)$_3$CO] in a mixture of 25 mL of acetone and 25 mL of water. This should be prepared immediately before use.

g) Standard chromium solution - Dissolve 0.0566 g of potassium dichromate ($K_2Cr_3O_7$) ll one litre of water. One millilitre of this solution contains 0.02 mg of chromium,

h) Sucrose

G-2.4.2 Procedure

Weigh 1.0g of the sample into a quartz dish. Cbar the material. raising the temperature slowly. Allow to cool. add 10 mL of the magnesium nitrate solution and evaporate, heating slowly until no more nitrous vapour evolves. Heat the material in an oven at 600±2°C until all black particles have disappeared (30 to 60 minutes). Dissolve the residue by adding 10 ml of the strong sulphuric acid solution and 20 ml of water. Heat on a water-bath for about 5 minutes.

Add 0.5 mL of the potassium permanganate solution, cover with a watch-glass and heat on a water-bath for about 20 minutes. Add more potassium permanganate, if the solution decolourizes, add sodium azide solution, one drops every 10 seconds, until the excess potassium permanganate has been removed. (Avoid excess of sodium azide, 2 drops are usually sufficient.) Cool the solution in running water and filler, if manganese dioxide is evident. Transfer the solution to a 50-mL graduated flask. Add 2.5 mL of sodium dihydrophosphate and 2mL of diphenylcarbazide solution and fill to the mark with water. Measure the extinction at 540 nm, 30 minutes after adding the diphenylcarbazide solution, A blank with the last two reagents (sodium dihydrophosphate and diphenyl-carbazide solutions) should show no colour or only a slight purple colour. At the same time, run a parallel test with 1.00 ml of the standard chromium solution and a few milligrams of sucrose placed in a second quartz dish. Treat the mixture exactly as the sample and measure the extinction at the same wavelength.

Calculate the chromium content of the sample from the two extinction values observed.