

**REPORT
of
ANALYSIS OF PESTICIDE RESIDUES**

**in
SOFT DRINK SAMPLES
sent by
DIRECTORATE GENERAL OF HEALTH
SERVICES, NEW DELHI
to
CFL, Mysore**

**Dated
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**FROM
CENTRAL FOOD LABORATORY
AT
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MYSORE - 570 013**

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INTRODUCTION

Central Food Laboratory (CFL) at Central Food Technological Research Institute (CFTRI), Mysore is a ISO 9001-2000 certified and NABL accredited laboratory under aegis Council of Scientific and Industrial Research (CSIR), New Delhi. The CFL is an appellate body under DGHS. Recently the CFL has acquired its expertise and training in human resource development to address the analytical aspects of contaminants like Pesticides, Heavy metals, aflatoxins, microbial pathogens and toxins. This has helped in carrying out training of participants from customs, industries, Public health laboratories, Public analysts and participants from other countries. This laboratory has state of art analytical facility including GC with ECD, NPD, FPD, HPLC with DAD, VWD, FLD and GC-MS with highly experienced and qualified personnel to carry out the specialized fields of testing.

MATERIAL AND METHODS

Sampling:

12 brands of soft drink samples from Jai Drinks Pvt. Ltd., Jaipur, Varun Beverages Ltd., Jodhpur and Mathura, Hindusthan Cola Beverages, Gaziabad. Samples were sent by the Directorate General of Health Services, New Delhi to CFL, CFTRI, Mysore for analysis.

Directorate General of Health Services (DGHS), New Delhi had sent duplicate samples 500 ml each of 12 brands of soft drinks from Delhi for the analysis of pesticide residues especially for the ones reported by CSE, New Delhi.

Equipment:

HP6890 Gas chromatograph fitted with Ni63 μ -ECD, NPD and Shimadzu GC 2010 with FPD was used for the quantification of organochlorine and organophosphorus insecticides. The columns used were HP 50+ equivalent to DB-17 and BPX 5 equivalent to DB-5 were used.

Perkin Elmer Turbomass Gold GC-MS connected to Autosystem XL GC was used for confirmation.

GC-MS METHODOLOGY

GC-MS analyses were carried out using Perkin Elmer Autosystem XL Gas Chromatograph coupled with Turbo Mass Gold spectrometer. Selected Ion Monitoring (SIM) technique was employed for the analysis of a mixture of standards (0.05 ppb each prepared in 1 ml hexane) as well as analytical samples for the confirmation of the likely presence of pesticides and also for the determination of their levels in the samples. For each analyte, five most abundant and characteristic peaks ($m/z > 100$) were selected for monitoring.

GC-MS analytical conditions employed for the analysis are as follows:

Inj Temp: 200°C

Det Temp: 225°C

El: 70 eV

Injection volume: 1 µl direct injection

Carrier gas: Helium; 1 ml/min.

Column: Elite-5 (Cross bond 5% diphenyl-95% dimethyl polysiloxane); 30 m, 0.25 mm i.d., 0.25 µm film thickness

Temp. Program: 180°(10)/5°(210°C)

Solvents:

All the solvents used like Methylene chloride, Hexane, Acetone used were of HPLC grade from E-Merck.

Chemicals:

Pesticide Certified reference standards were obtained from Sigma Chemicals, USA.

Sample extraction:

The samples were analyzed using EPA method 8081A for organochlorines by gas chromatography and EPA Methods 8141 A for organophosphorus compounds.

Cleanup:

Cleanup was done by EPA method 3620B using florisil activated at 130°C overnight and cooled in a desiccator before use.

Sample analysis:

The 12 brands of soft drinks were analyzed for organochlorine insecticides focus namely HCH isomers (alfa, gamma, beta and delta), DDT and metabolites (pp DDT, op DDT, DDE and DDD), Endosulfan I, II and sulfate, Heptachlor, Heptachlor epoxide, chlordane organophosphorus insecticide like Methylparathion, Chlorpyrifos, Fenitrothion and Malathion.

Calculation:

All calculations were done as described in USEPA/AOAC method.

Recovery:

Recovery experiments were done in all the twelve brands of soft drinks sent to the laboratory for analysis spiking with 0.1 ppb of pesticides. The recovery was greater than 90%.

Confirmation and quantification:

Confirmation of the pesticide detected were carried out by dual column technique using HP 50+ and BPX 5 columns and by GC-MS.

RESULTS

A total of 12 brands of soft drinks were tested for organochlorine insecticides and 4 organophosphorus insecticides.

Organochlorine insecticide residues: Lindane was found to be present in 100% of the samples. 33% of the samples were exceeding the EEC limit of 0.1ppb. DDT and metabolites were present in 58% of the samples analyzed. DDT and metabolites exceeded the EEC limit in 58%. The lindane exceeded the limit from 1.1 to 1.4 times the limit. DDT and metabolites exceeded the limit 1.8 to 12.4 times the EEC limit.

Organophosphorus insecticide residues: Chlorpyrifos was present in 100% of the samples. Chlorpyrifos residue exceeded the limit in 75% of the samples. The chlorpyrifos residue exceeded the limit by 3.9 to 7.6 times.

The total insecticides (OC and OP) exceeded the limit in 75% of the samples and total residue was 1.16 to 5.2 times the 0.5ppb EU limit.

By going through the CSE report that the CSE followed confirmatory tests like dual column and TLC. Therefore to substantiate the presence of these insecticides GC-MS was used in addition to dual column technique and spiking

experiments. As the samples analyzed at CFL, CFTRI, Mysore was entirely from a different batch than the CSE samples, the results obtained are not comparable with the results of CSE.

CONCLUSION

- Lindane was present in 100% of the samples. The concentration ranged from 0.000008 to 0.00014 mg/L. 33% of the samples exceeded EEC limit in the range 1.1 to 1.4 times the EEC limit (See Annexure 1 to 4 for details).
- DDT and metabolites were present in 58% of the samples ranging from 0.00018 to 0.00124 mg/L. DDT and metabolites exceeded the limit in the range 1.8 -12.4 times the EEC limit. (See Annexure 1 to 4 for details).
- Chlorpyrifos was present in 100% of the samples analyzed and it exceeded the limit in 75% of the samples. Chlorpyrifos residue exceeded the limit by 3.9 to 7.8 times the EEC limit (See Annexure 1 to 4 for details).
- Malathion was not detected in any of the 12 samples.

Organochlorine Pesticide Residues in Soft Drinks (mg/L)

Sl. No.	Brand Name	α -HCH	β -HCH	γ -HCH	δ -HCH	Total HCH	DDT	DDE	DDD	Total DDT + Metabolites	Total Organochlorines (OC)
1	Limca	ND	ND	0.000009	ND	0.000009	ND	ND	ND	ND	0.000009
2	Diet Pepsi	ND	ND	0.000046	ND	0.000046	0.00018 (1.8)	ND	ND	0.00018	0.000226
3	Pepsi	ND	ND	0.000008	ND	0.000008	ND	ND	ND	ND	0.000008
4	7Up	0.0001	ND	0.000094	ND	0.000194	ND	ND	ND	ND	0.000194
5	Fanta	0.0002 (2)	ND	0.00013 (1.3)	ND	0.00033	ND	ND	ND	ND	0.00033
6	Mirinda (Lemon Flavour)	0.0004 (4)	ND	0.00005	ND	0.00045	0.00022 (2.2)	0.00066 (6.6)	ND	0.00088	0.00133
7	Mountain Dew	0.0003 (3)	ND	0.00009	ND	0.00039	ND	ND	ND	ND	0.00039
8	Thums Up	0.0003 (3)	ND	0.00007	ND	0.00037	ND	0.00048 (4.8)	ND	0.00048	0.00085

Contd....

Organochlorine Pesticide Residues in Soft Drinks (mg/L) (Contd..)

Sl. No.	Brand Name	α -HCH	β -HCH	γ -HCH	δ -HCH	Total HCH	DDT	DDE	DDD	Total DDT + Metabolites	Total Organochlorines (OC)
9	Coca Cola	0.0003 (3)	ND	0.00014 (1.4)	ND	0.00044	ND	0.00088 (8.8)	ND	0.00088	0.00132
10	Mirinda (Orange Flavour)	0.0003 (3)	ND	0.00011 (1.1)	ND	0.00041	ND	0.0008 (8)	ND	0.0008	0.00121
11	Sprite	0.0003 (3)	ND	0.00003	ND	0.00033	ND	0.0008 (8)	ND	0.0008	0.00113
12	Blue Pepsi	0.0005 (5)	ND	0.00013 (1.3)	ND	0.00063	ND	0.00124 (12.4)	ND	0.00124	0.00187

ND = Not detected

Note : Figure given in the parenthesis indicates number folds higher than European Norms for Packaged Drinking Water (0.0001mg/L for individual pesticides and 0.0005mg/L for Total Pesticides)

Repeatability, Reproducibility and Trueness of the results as per the Alinorm 3/24 of Codex Alimentarius commission i.e. "Report of the 34th session of the Codex Committee on Pesticide Residues (The Hague, The Netherlands, 13-18 May - 2002) as reported in the Joint FAO/WHO Food standard programme of CAC, 30th June - 5th July, 2003.

Organophosphorous Pesticide Residues in Soft Drinks (mg/L)

Sl. No.	Brand Name	Chlorpyrifos	Malthion	Total Organo phosphorous (OP)	Total OC + OP
1	Limca	0.00002	ND	0.00002	0.000029
2	Diet Pepsi	0.00004	ND	0.00004	0.000266
3	Pepsi	0.000017	ND	0.000017	0.000025
4	7Up	0.00039 (3.9)	ND	0.00039	0.000584 (1.6)
5	Fanta	0.00054 (5.4)	ND	0.00054	0.00087 (1.74)
6	Mirinda (Lemon Flavour)	0.00078 (7.8)	ND	0.00078	0.00211 (4.2)
7	Mountain Dew	0.00061 (6.1)	ND	0.00061	0.00102 (2.0)
8	Thums Up	0.00063 (6.3)	ND	0.00063	0.00100 (2.0)

Contd....

Organophosphorous Pesticide Residues in Soft Drinks (mg/L) (Contd..)

Sl. No.	Brand Name	Chlorpyrifos	Malthion	Total Organo phosphorous (OP)	Total OC + OP
9	Coca Cola	0.00070 (7.0)	ND	0.00070	0.002 (4.0)
10	Mirinda (Orange Flavour)	0.00050 (5.0)	ND	0.00050	0.00171 (3.4)
11	Sprite	0.000498 (4.9)	ND	0.000498	0.001628 (3.2)
12	Blue Pepsi	0.00076 (7.6)	ND	0.00076	0.00263 (5.2)

ND = Not detected

Note : Figure given in the parenthesis indicates number folds higher than European Norms for Packaged Drinking Water (0.0001mg/L for individual pesticides and 0.0005mg/L for Total Pesticides)

Repeatability, Reproducibility and Trueness of the results as per the Alinorm 3/24 of Codex Alimentarius commission i.e. "Report of the 34th session of the Codex Committee on Pesticide Residues (The Hague, The Netherlands, 13-18 May - 2002) as reported in the Joint FAO/WHO Food standard programme of CAC, 30th June - 5th July, 2003.

DETAILS OF SAMPLES RECEIVED BY CFL (CFTRI), MYSORE

Number of samples received : 12 Nos. (2 x 500ml each)

Received Date : 09/08/2003

Sl. No.	Name of the Product	Name of the manufacturer	Date of manufacture	Best Before Date	Batch No.
1	Limca	Coca Cola Beverages Pvt. Ltd. Tehsil Hapur Dist. Gaziabad	11/07/03	2 Months from the date of manufacture	1649
2	Diet Pepsi	Jai Drinks Pvt. Ltd., Jawahar Lal Nehru Marg, Jaipur	28/07/03	2 Months from the date of manufacture	OP.03.17
3	Pepsi	Jai Drinks Pvt. Ltd., Jawahar Lal Nehru Marg, Jaipur	09/07/03	3 Months from the date of manufacture	P.03.164
4	7Up	Jai Drinks Pvt. Ltd., Jawahar Lal Nehru Marg, Jaipur	20/06/03	3 Months from the date of manufacture	No. S.03.12
5	Fanta	Bottled by Hindustan Coca cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad	12/06/03	1.5 Months from the date of manufacture	B.N.1373
6	Mirinda (Lemon Flavour)	Jai Drinks Pvt. Ltd., Jawahar Lal Nehru Marg, Jaipur	30/05/03	3 Months from the date of manufacture	ML. 03.13

Contd.....

CFL (CFTRI), MYSORE

Sl. No.	Name of the Product	Name of the manufacturer	Date of manufacture	Best Before Date	Batch No.
7	Mountain Dew	Jai Drinks Pvt. Ltd., Jawahar Lal Nehru Marg, Jaipur	10/07/03	3 Months from the date of manufacture	MD 03.33
8	Thums Up	Bottled by Hindustan Coca cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad	04/06/03	1.5 Months from the date of manufacture	1276
9	Coca Cola	Bottled by Hindustan Coca cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad	18/07/03	2 Months from the date of manufacture	1677
10	Mirinda (Orange Flavour)	Jai Drinks Pvt. Ltd., Jawaharlal Nehru Marg, Jaipur	22/07/03	3 Months from the date of manufacture	MO. 03.56
11	Sprite	Bottled by Hindustan Coca cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad	29/05/03	2 Months from the date of manufacture	1195
12	Blue Pepsi	Jai Drinks Pvt. Ltd., Jawaharlal Nehru Marg, Jaipur	02/04/03 16/03/03	3 Months from the date of manufacture	PB. 03.18 PB. 03.17

Comparison of the results* of CFL (CFTRI), Mysore and CSE, New Delhi

Sl. No.	Brand	CFL (CFTRI), Mysore			CSE, New Delhi		
		Batch No.	Total Pesticide Residues OC + OP (mg/L)	No. of folds higher than EEC Limits	Batch No.	Total Pesticide Residues OC + OP (mg/L)	No. of folds higher than EEC Limits
1	Limca	1649	0.000029	Below EEC limits	BN 747 BN 757 BN 753	0.0148	30
2	Diet Pepsi	OP.03.17	0.000266	Below EEC limits	DP.03.11.13:21 DP.03.9.14:25 DP.03.11.18:21	0.0071	14
3	Pepsi	P.03.164	0.000025	Below EEC limits	PN- 99 P.03.76.06:06 P.03.054.01:50	0.0187	37
4	7Up	No. S.03.12	0.000584	1.6	S.03.02.19.06 S.03.02.00.43 S.03.02.20:24	0.0166	33
5	Fanta	B.N.1373	0.00087	1.7	BN 780 BN 776 BN 537	0.0214	43
6	Mirinda (Lemon Flavour)	ML. 03.13	0.00211	4.2	ML.03.9.21:21 ML.03.07.15:23 ML.03.13.17.43	0.0352	70

Contd.

CFL (CFTRI), MYSORE

Sl. No.	Brand	CFL (CFTRI), Mysore			CSE, New Delhi		
		Batch No.	Total Pesticide Residues OC + OP (mg/L)	No. of times higher than EEC Limits	Batch No.	Total Pesticide Residues OC + OP (mg/L)	No. of times higher than EEC Limits
7	Mountain Dew	MD 03.33	0.00102	2.0	MO.03.19.10:00 MO.03.13.15:09 MO.03.26.17:12	0.0141	28
8	Thums Up	1276	0.00100	2.0	BN 720 BN 727 BN 525	0.0111	22
9	Coca Cola	1677	0.002	4.0	BN 724 BN 512 BN 738	0.0223	45
10	Mirinda (Orange Flavour)	MO. 03.56	0.00171	3.4	MO.03.24.22:08 MO.03.27.15:22 L3	0.0196	39
11	Sprite	1195	0.001628	3.2	BN 787 BN 796 BN 791	0.0055	11
12	Blue Pepsi	PB. 03.18 PB. 03.17	0.00263	5.2	PB.03.19.13:57 PB.03.18.21:20 PB.03.19.21:28	0.0147	29

OC = Organochlorine; OP = Organophosphorus

*The batch Nos. of the samples of CFL (CFTRI), Mysore and CSE, New Delhi are not the same. However, only the brand name is indicated for comparison and Batch Nos. are also indicated.