

Response to the “Report of the Expert Committee to review the CSE report on analysis of pesticide residues in soft drinks” set up by the Ministry of Health and Family Welfare

By Centre for Science and Environment, New Delhi

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The Centre for Science and Environment set up its Pollution Monitoring Laboratory in 2001 to undertake studies in the public interest. The laboratory has done studies on endosulfan pesticides in Padre village in Kerala, pesticide residues in drinking water and vegetables in Delhi, pesticide residues in packaged drinking water sold in Delhi and Mumbai, pesticide residues in carbonated beverages and heavy metals in the environmental samples in the vicinity of Pali village in Rajasthan. In addition, it has a number of ongoing studies.

In 2003, its report on pesticide residues in soft drinks had lead to the formation of the Joint Parliamentary Committee (JPC). The terms of reference of the 15 member JPC were to investigate:

- a. whether the recent findings of the Centre for Science and Environment regarding pesticide residues in soft drinks are correct or not;
- b. to suggest criteria for evolving suitable safety standards for soft drinks, fruit juices and other beverages where water is the main constituent.

The JPC report was submitted in February 2004, after extensive work to check the veracity, working and methodology of the CSE laboratory. The JPC reviewed sampling methodologies, equipments, methods of analysis, accreditation status, internal verification systems, results etc. The JPC, after this extensive technical and scientific deliberations endorsed CSE study and stated: “the committee, find that the CSE findings are correct on the presence of pesticide residues in carbonated water strictly in respect of the 36 samples of 12 brand names analysed by them.”

1. Expert Committee: The report does not provide details about the following points, which are required for confirmatory interpretation of quantum results.

1.1 Expert Committee: Quantity and volume of sample collected from market for analysis not given in the report? For tests like the one done on soft drinks by CSE, 3 litre samples is required.

CSE response: The information is given in the report, Analysis of Pesticide Residues in Soft Drinks, CSE 2006 (see page 9 and Annexure 1). The report gives details of the place where the sample was collected, the place of manufacture of the sample, the batch number and the date of expiry. CSE collected three bottles of each sample (total 171 bottles of soft drinks – 57 x 3). Volumes of samples ranged from 600 ml to 1 litre (for some sample 3 bottles of 600 ml were collected and for some three bottles of 1 litre were collected), depending on the availability at that time in the market. The tests were conducted three times (triplicate) for each sample. In each test 500 ml sample were extracted for residue analysis. The quantity of sample (500 ml) extracted was sufficient to give clear quantifiable results about pesticide residues in soft drinks.

The assertion that 1 litre sample is required for each test is scientifically untenable because if clear quantifiable results are attainable in 500 ml, then there is no need to increase the volume of sample. Higher volume of sample is used when the quantity of pesticide in the sample is very difficult to detect and quantify, which was not the case in CSE study on pesticide residues in soft drinks.

Sample quantity referred in the prescribed USEPA method is approximate. It is only indicative and not rigid. It is well understood that the laboratory analysts can optimise parameters to get the best resolution. This issue was also discussed in detail with the scientific experts of the Joint Parliamentary Committee.

It is rather strange that the expert committee has chosen to raise this objection, when the health ministry own laboratory had used samples of 500 ml to test soft drinks. In 2003, CFL-Mysore and CFL-Kolkata had used 500 ml sample of soft drink to test for the Ministry of Health and Family Welfare. Their methods were scrutinised and found valid by the Joint Parliamentary Committee (see page 4 and 6 of the Report of the joint committee on pesticide residues in and safety standards for soft drinks, fruit juice and other beverages, Lok Sabha Secretariat, New Delhi, February 2004).

1.2. Expert Committee: Final volume of the cleaned-up extracts for GC-ECD/NPD analysis not given in the report.

CSE response: The details of the method used for sample extraction and clean up are given on page 10 of the CSE report. Final volume of cleaned-up extracts is generally not given in the scientific reports.

As per the laid down scientific practice, the final volume of the extract is arrived at by the analyst depending upon the sensitivity for the compounds of interest. In this study for organochlorines analysed by GC-ECD the final volume of the clean up extract was 2.0 ml and for organophosphorus pesticides analysed by GC-NPD the final volume of the extract was 0.5 ml.

1.3 Expert Committee: Details of fortification levels (spike levels) for the recovery experiments and limit of quantification not given in the report.

CSE response: The details are given in the report (see page 11: section 6.8). Information is provided clearly of the method of fortification and the recovery experiments done. The report also clearly states the percentage recovery and retention time for both organochlorines and organophosphorus pesticides. Further details are given in Table 1 and Table 2 below.

Table: 1 Spike levels and limit of quantification for organochlorine pesticides

	Spike levels (ppb)	S1	S2	S3	S4	S5	S6	S7	Mean (ppb)	Standard Deviation (SD)	Recovery (%)	LOQ (in ppb) 10 x SD	MDL (ppb) SD x t-value	S/N MEAN/SD
Alpha-HCH	0.02	0.0188	0.0172	0.0159	0.0169	0.0179	0.0198	0.0140	0.0172	0.0019	86.1	0.019	0.0060	9.0
Beta-HCH	0.02	0.0176	0.0188	0.0157	0.0198	0.0200	0.0170	0.0200	0.0184	0.0017	92.0	0.017	0.0053	10.8
Gamma-HCH	0.01	0.0102	0.0092	0.0110	0.0124	0.0120	0.0110	0.0130	0.0113	0.0013	112.6	0.013	0.0041	8.7
Delta-HCH	0.02	0.0210	0.0199	0.0185	0.0179	0.0199	0.0189	0.0152	0.0187	0.0019	93.7	0.019	0.0059	10.0
Alpha-Endosulfan	0.02	0.0189	0.0189	0.0186	0.0156	0.0186	0.0198	0.0166	0.0181	0.0015	90.7	0.015	0.0046	12.4
Beta-Endosulfan	0.02	0.0175	0.0197	0.0160	0.0199	0.0189	0.0196	0.0199	0.0188	0.0015	93.9	0.015	0.0047	12.6
Endosulfan sulphate	0.02	0.0158	0.0126	0.0139	0.0188	0.0169	0.0199	0.0197	0.0168	0.0029	83.9	0.029	0.0090	5.9
Aldrin	0.02	0.0196	0.0188	0.0172	0.0157	0.0169	0.0198	0.0178	0.0179	0.0015	89.7	0.015	0.0047	12.1
Dieldrin	0.02	0.0176	0.0137	0.0189	0.0179	0.0147	0.0180	0.0175	0.0169	0.0019	84.4	0.019	0.0060	8.8
Heptachlor	0.02	0.0191	0.0188	0.0186	0.0174	0.0188	0.0138	0.0190	0.0179	0.0019	89.5	0.010	0.0060	9.4

Chlordane	0.02	0.0188	0.0190	0.0124	0.0176	0.0180	0.0189	0.0180	0.0175	0.0023	87.4	0.020	0.0073	7.5
DDT	0.05	0.0480	0.0400	0.0370	0.0450	0.0500	0.0380	0.0450	0.0433	0.0050	86.6	0.010	0.0158	8.6
DDD	0.05	0.0476	0.0457	0.0424	0.0380	0.0490	0.0469	0.0428	0.0446	0.0038	89.2	0.010	0.0120	11.7
DDE	0.05	0.0437	0.0490	0.0463	0.0885	0.0480	0.0457	0.0480	0.0527	0.0159	105.4	0.010	0.0499	3.3

Note: No. of observations=7 with six degrees of freedom . The students t- value for 7 replicates and 6 degrees of freedom is 3.143

Table: 2 Spike levels and limit of quantification for organophosphorous pesticides

	Spike levels (ppb)	S1	S2	S3	S4	S5	S6	S7	Mean (ppb)	Standard Deviation (SD)	Recovery (%)	LOQ (in ppb) 10 x SD	MDL (ppb) SD x t-value	S/N MEAN/SD
Monocrotofos	1	0.8785	0.7864	0.7936	0.7358	0.6946	0.7956	0.7584	0.7776	0.0575	77.8	0.575	0.1807	13.5
Chlorpyrifos	0.2	0.1985	0.1875	0.1968	0.1788	0.1990	0.1552	0.1981	0.1877	0.0162	93.9	0.162	0.0509	11.6
Malathion	0.5	0.5100	0.4896	0.4263	0.4963	0.4963	0.4756	0.0429	0.4196	0.1683	83.9	1.683	0.5289	2.5
Dichlorvos	0.2	0.1897	0.1863	0.1578	0.1996	0.1884	0.1963	0.1663	0.1835	0.0155	91.7	0.155	0.0489	11.8
Acephate	0.5	0.4796	0.4365	0.4956	0.4236	0.3689	0.4756	0.4963	0.4537	0.0468	90.7	0.468	0.1469	9.7
Phorate	0.2	0.1799	0.1563	0.1669	0.1839	0.1796	0.1996	0.1606	0.1753	0.0150	87.6	0.150	0.0472	11.7
Diazinon	0.2	0.1789	0.0169	0.1479	0.1863	0.1899	0.1589	0.1796	0.1512	0.0611	75.6	0.611	0.1921	2.5
Fenthion	0.2	0.1789	0.1589	0.0180	0.1586	0.1498	0.1756	0.1863	0.1466	0.0582	73.3	0.582	0.1829	2.5
Dimethoate	0.5	0.4869	0.4169	0.4693	0.4798	0.3699	0.4256	0.4712	0.4457	0.0429	89.1	0.429	0.1349	10.4
Phenthoate	0.5	0.4236	0.3568	0.4796	0.4263	0.4258	0.4639	0.4693	0.4350	0.0416	87.0	0.416	0.1309	10.4
Profenofos	1	0.8526	0.7963	0.8859	0.6507	0.7656	0.8523	0.7693	0.7961	0.0787	79.6	0.787	0.2475	10.1
Ethion	0.2	0.1852	0.1893	0.1796	0.1796	0.1769	0.1496	0.1369	0.1710	0.0197	85.5	0.197	0.0621	8.7

Note: No. of observations=7 with six degrees of freedom. The students t- value for 7 replicates and 6 degrees of freedom is 3.143

1.4 Expert Committee: Limit of detection (LOD) of individual pesticides on GC_ECD/NPD not given in the report.

CSE response: The CSE laboratory has its in-house checks and Good Laboratory Practices (GLP), which are followed diligently. A detailed study was conducted in the Lab and LOD of individual pesticides was evaluated by externally calibrating the GC using 5 standards of different concentrations. Seven aliquots of soft drink samples were fortified with low concentrations of pesticides. Number of observations is equal to the number of replicates, 7 with six degrees of freedom. The extracts were concentrated to 0.5 – 2.0 ml and were analysed by gas chromatography using appropriate instrument parameters. Method Detection Limit (Limit of detection) was calculated by multiplying

the Standard Deviation (SD) by correct students t value. The student's t value for 7 replicates and 6 degrees of freedom is 3.143.

$$\text{MDL} = (\text{SD}) \times (\text{t-value})$$

$$\text{LOQ} = 10 \times (\text{SD})$$

Method detection limit was verified using points given below:

CHECK	Requirement	Criteria –Meets/Not Meets
Spike level	MDL*10>spike	OK, Meets criteria
Spike level	MDL< Spike	OK, Meets criteria
MDL	0.1 ppb	OK
Average percentage recovery for pesticides reported as confirmed	80- 120%	OK, Meets criteria
Signal/Noise (average/std devn)	Between 2.5- 10	OK, Meets criteria

1.5 Expert Committee: Limit of detection of GC-MS for the confirmation of individual pesticides in market samples.

CSE response: The expert committee has misinterpreted the CSE report. The report clearly states that the GC-MS analysis was done for confirmation of pesticides. The quantification was done for all 57 samples by GC-ECD and GC-NPD.

GC-MS analysis was done only for three samples which contained all the pesticides reported. Limit of detection was not evaluated for GC-MS as it was used for only confirmation and not quantification.

There are two aspects of the CSE report: quantitative (identification, detection and quantification of amount of pesticides present) and qualitative (re-confirmation of pesticide molecules present). In 2003 study, CSE had undertaken the quantitative study, with GC-ECD/NPD, which were found to be correct by the Joint Parliamentary Committee. JPC, however, had recommended that CSE should also undertake qualitative analysis in its future studies.

In 2006, like in 2003, for quantitative analysis of organochlorines, CSE has used Gas Chromatograph (Thermoquest-Trace GC) equipped with the ⁶³ Ni selective electron-capture detector. The capillary column used was DB- 1701 (14%-Cyanopropyl-phenyl)-

methylpolysiloxane (length 30m, ID 0.25 mm and film 0.25 μ m). For the organophosphorus pesticides, CSE has used Gas Chromatograph (Nucon -5765 series equipped with Nitrogen Phosphorus detector) and a capillary column used was DB- 210 (50% trifluoro propyl polysiloxane, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25 μ m). The samples were analysed by using EPA Method 8081A for organochlorines by Gas chromatography and EPA Method 8141A for organophosphorus compounds by gas chromatography: Capillary column technique. Cleanup was done by EPA Method 3620B- Florisil Clean up by Column Chromatography. Please note that all this information is available in the report.

For qualitative identification and confirmation of the pesticides, CSE this time used Gas Chromatograph equipped with Mass Spectrometer (GC-MS) - model Thermo finnigan Polaris Q Ion Trap GC MS at 70eV in), in Selective Ion Monitoring (SIM) mode. **Re-confirmation of the pesticide molecules was done by running the sample and the standard under same condition. Standard was run only to obtain a fragmentation pattern; confirmation was done by evaluation of mass spectral pattern of the sample with the library match within pesticide spectral library. The primary ion (m/z) should be supported by two secondary ions between the reference and the sample spectrum.**

The characteristic ions from the reference mass spectrum are defined as three ions of greatest relative intensity or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. {Reference: EPA Method 8270 C- Semi volatile organic compounds by Gas Chromatography /Mass Spectrometry (GC/MS)}.

GC-MS was used only to confirm the presence of pesticides in the sample. It was not used to quantify the amount of pesticide residues present in the samples. Therefore, the question is infructuous.

Moreover, it is important to note that identification of pesticides in soft drink was unequivocally confirmed by the quantitative analysis itself. Identifications were confirmed by spiking the sample with known standard only to confirm the unknown. Moreover, the identifications were crosschecked with another column- DB-5 coated with 5% diphenyl and 95% dimethylpolysiloxane of different polarity. Elution pattern was

different from the elution pattern in DB-17. **This same method of confirmation of pesticide molecules in the soft drink was also done by almost all labs in 2003 (including CFL-Calcutta and CPCB, New Delhi).**

1.6 Expert Committee: GC-MS chromatograms of various experiments not given in the report.

CSE response: All GC-ECD/NPD chromatograms and GC-MS spectra are documented and preserved and are available for public scrutiny. In 2003, the Joint Parliamentary Committee had scrutinised all such information and found it in order.

1.7 Expert Committee: Nature of laboratory fortified sample matrix not given in the report

CSE response: It is given on page 11 of the CSE report. Laboratory fortified matrix sample is a matrix which is close to or identical to that of the sample of interest. In our case, a sample of soft drink was taken and a known quantity of analyte was added in the laboratory in ppb range. This laboratory fortified matrix was analysed exactly like a sample and its purpose was to determine whether the sample matrix contributes bias to the analytical results. About 10% of the samples were fortified with a known concentration of pesticides and percent recovery was calculated. Extraction was done as per USEPA 8081A for organochlorines and 8141A for organophosphorus and clean up was done as per USEPA- 3620 B Florosil clean up by column chromatography. The recovery of the 15 organochlorine and 13 organophosphorus pesticides over the background values obtained from unfortified samples *were more than 80 per cent in most of the cases which showed that the sample matrix analysis was within the recovery range criterion (60- 120%) with good laboratory practices.*

1.8 Expert Committee: Total ion chromatogram (GC-MS) of various samples not given in the report.

CSE response: The GC-MS spectra is given in Annexure III of the report. The expert committee has misinterpreted the methodology used. GC-MS analysis was done for three samples and all pesticides detected in GC-ECD/NPD analysis was confirmed by these three GC-MS analysis. The total ion chromatograms of GC-MS analysis have been properly documented and are available for public scrutiny. Quantification was done for all 57 samples by GC-ECD and GC-NPD; the chromatograms for these tests are also available for public scrutiny.

1.9 Expert Committee: Source of recovery sample not given in report

CSE response: Please see page 11 of the report for details on the recovery experiments. Source of recovery sample was not provided in the report because it is not required. However, it is given below as it has been asked for.

A matrix reference material which is a homogenous material containing known concentration of one or more analytes is used to check the performance of an analytical method. When no suitable reference material is available, the practice is to add a known amount of pure analyte to a matrix of same composition as the samples or those containing a very low natural amount of analyte (soft drink). This is called spiking. Spiked samples are normally used in method validation to help identify matrix effects and determine the recovery of an analyte or the selectivity and sensitivity of the method. The source of recovery sample in CSE study was a soft drink sample spiked with known amounts of analytes. The results of the recovery experiments are in the CSE report (pl. see 11 for table on percent recovery and retention time for individual pesticides).

2. Expert Committee: Comparison of GC-MS samples with library hits has been done at 5 ppm whereas the confirmation of the contaminants in various samples has been done at ppb level. Most of the characteristic fragment ions are not recorded despite the fact the various pesticide contaminants have been claimed to be present in market samples. Therefore, the residue data reported based on GC-Mass confirmation is inconclusive.

CSE response: The expert committee has completely misinterpreted the report. The GC-MS has not been used for quantification of the pesticides but only for confirmation of

identity of pesticide molecules. In fact, the GC-MS has merely assisted us confirming what was found in 2003, not just by the CSE laboratory but also by government laboratories. The presence of pesticide was detected with GC-ECD and GC-NPD. It must be recognized that few laboratories in the country have access to GC-MS and therefore, the bulk of pesticide residue testing done in India is done only on GC-ECD and GC-NPD. **The tests done on the GC-MS were to reconfirm what had already been found by GC-ECD and GC-NPD. Therefore, the expert committee view that the report is “inconclusive” is completely scientifically untenable.**

The presence of pesticide residues detected in the samples by GC-ECD and GC-NPD were confirmed by GC-MS, model Finnigan Polaris Q Ion trap GC/MSⁿ with EI ionization (70eV), in Selective Ion Monitoring (SIM) mode. The sample and standards were injected using a programmable temperature injector (PTV) with a cold solvent splitless injection of 2 micro litre. **GC-MS was not used for quantification of the samples.** Therefore, the 5 ppm level standard was injected into GC-MS only for qualitative analysis, to estimate the fragmentation pattern and relative retention time for the pesticide to be confirmed and not for quantification.

For quantification GC-ECD and GC-NPD were used for organochlorines and organophosphorus pesticides respectively. Calibration standard corresponding to the expected range of concentration found in real samples was used for quantification. GC system was calibrated using *external standard technique*. Calibration standard at five concentration levels for each standard of interest was prepared from the stock standard solution as follows

Standard solution preparation

Stock standard solution: A concentrated solution containing a single certified standard was prepared (99 per cent purity). Stock standard solution was used to prepare primary dilution standards.

Primary dilution standard solution: A solution of standard prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solution.

Calibration standard was prepared from the primary dilution standard solution and it was used to calibrate the instrument response with respect to analyte concentration at 5 different concentrations 0.5, 0.2, 0.1, 0.05 and 0.01 ppm in case of GC-ECD and 2.0, 1.0, 0.5, 0.1 and 0.05 ppm in case of GC-NPD.

3.1 Expert Committee: The prevalence of different isomers of HCH are in contradiction to their biological nature. Though, beta HCH is more persistent, surprisingly most of the 57 samples do not show the presence of beta isomer.

CSE response: It is scientifically wrong to link the presence of beta-HCH in soft drink samples with that of its persistent nature. Many more pesticides are persistent and they are not always found in all food, water and environmental samples. It is, therefore, not necessary that beta-HCH should be found in all the soft drink samples. CSE has found beta-HCH in 14 per cent of the soft drink samples.

3.2 Expert Committee: As regard to delta isomer which is rarely encountered was detected in high concentration in all the samples which is contrary to the normal findings.

CSE response: This again is scientifically untenable and factually incorrect question. Delta-HCH is a persistent isomer of HCH and therefore its presence in the environment shouldn't be surprising. It is precisely because of this that government's own labs, and various research organisations all over the country, have also found delta-HCH in various samples. Residues of delta HCH have been found in numerous samples of milk, drinking water, pond water, vegetables, soil samples and even in rainwater samples from various parts of the country. Delta HCH was found in samples from Bhatinda, Trivandrum, Hissar, Sirsa, Rohtak, Delhi, Shahjahanpur, Hardwar and other parts of India. If CSE's findings are "contradictory" then so are of eminent government institutions like the Indian Council of Medical Research, the Indian Council of Agricultural Research and the Chandigarh-based Post Graduate Institute of Medical Education and Research (PGI). Questioning the presence of delta HCH in soft drink is therefore a scientifically untenable argument.

Some of the studies that have found Delta-HCH are reproduced below for the perusal of the expert committee:

- The Indian Council of Medical Research (ICMR) undertook a multicentric study from 1986 to 1991 to assess the magnitude of contamination of certain food commodities in rural and urban areas of the country. Its report published in 1993 — *Surveillance of Food Contaminants in India* — which was not made public, gives clear evidence about presence of delta HCH milk samples. A total of 2,205 milk samples from 12 states of the country were tested for pesticide residues. HCH was detected in 85 per cent samples. The percentage of samples exceeding the maximum residue limits (MRLs) was 21, 42 and 28 per cent in case of alpha, beta and delta HCH respectively.
- A study conducted by the PGI in 2004 on pesticide residues in food commodities and water in Bhatinda district of Punjab found delta HCH in both food commodities and water in high quantities. Delta HCH was found in both ground water and tap water. In vegetable samples also delta HCH were found.
- The result of pesticide residues in milk samples collected from different parts of Trivandrum district, Kerala indicated the contamination with different isomers of HCH. Delta HCH was present in two milk samples at 3 ppb and 9 ppb level. (Source: All India Coordinated Research Project on Pesticide Residues, Indian Council of Agricultural Research, February 2002).
- Delta-HCH was detected in pond water samples collected from rice growing areas of Hissar, Sirsa and Rohtak at mean levels of 0.4-1.0 ppb. (Source: Kumari B et al (1996) Bulletin of Environment Contamination Toxicology 57: 787-793)
- Delta-HCH was found in different brands of nine mineral water samples at mean level of 0.01-0.05 ppb. (Source: Om Prakash et al (2004), Current Science, Vol 87, No. 1)
- Residues of all isomers of HCH, including delta HCH, were detected in all soil samples taken from agricultural land nearby Delhi. Delta HCH was detected at mean levels of 0.08 ppb to 3.60 ppb in surface soil and at 0.07 ppb to 0.20 ppb in sub-surface soil. (Source: Om Prakash et al (2004), Current Science, Vol 87, No. 1)
- Delta-HCH detected in 22 pond water samples collected from 13 villages of district Shahjahanpur, Uttar Pradesh at mean levels of 0.11 to 0.17 ppb. (Source: Dua V.K et al (1996) Bulletin of Environment Contamination Toxicology 57: 568-574)
- Delta-HCH residues detected at a mean level of 0.018 ppb in rainwater samples from Hardwar, UP. (Source: Dua V.K et al (1994) Bulletin of Environment Contamination Toxicology 52: 797-801).

3.3 Expert Committee: Although the gamma isomer (Lindane) has been reported in the every sample, GC mass spectra of Lindane is not available.

CSE response: First of all, Lindane was identified and quantified by GC_ECD on the basis of retention time and peak area. The identity was further confirmed by GC-MS. Technical BHC is a mixture of a number of stereoisomerisms, principally alpha-BHC (55-70%), beta-BHC (5-14%), gamma-BHC (10-18%) and delta-BHC (6-10%). Stereoisomers have same structural formula but differ in arrangement of atoms or groups in space. The isomers differ in their axial-equatorial substitution pattern around the ring, only delta-HCH is chiral. There are two categories of stereoisomers -- enantiomers and diastereomers. Enantiomers are stereoisomers whose molecules are mirror images of each other and diastereomers are not mirror images of each other. All the isomers of HCH because of same structural formula have identical fragmentation pattern only differ in the intensity of ions. The gamma isomer of HCH has been confirmed by GC-MS. The library results identify gamma-HCH as beta-HCH. This can be seen by the GC-MS spectra of the standard, given in the report.

4. Expert Committee: Malathion undergoes hydrolysis in acidic medium of soft drinks. Hence, its residue in such a concentration is unlikely. GC-MS chromatogram also confirms absence of malathion.

CSE response: The same issue was raised and analysed in detail by the Joint Parliamentary Committee (JPC) in 2003-04: CSE's malathion findings withstood scientific scrutiny and were endorsed by the JPC (see page 19 and 20 of the Report of the joint committee on pesticide residues in and safety standards for soft drinks, fruit juice and other beverages, Lok Sabha Secretariat, New Delhi, February 2004).

It is to be noted that in 2003 CSE was not the only organization that found malathion in soft drinks. Central Pollution Control Board Laboratory conducted an independent analysis of samples of 6 brands of soft drinks collected from various markets in Delhi and detected Malathion in all the six samples tested by it.

After CSE Report, Directorate of Health Services, Government of Kerala had sent a sample each of Coca Cola and Pepsi of soft drink for analysis to Shriram Institute for Industrial Research, Bangalore which is one of the NABL accredited laboratory for testing pesticide residues in water. This laboratory had found 0.7 ppb of Malathion in the

sample of Pepsi and 9.9 ppb of Malathion in Coca Cola analysed by it. Protocol used by this laboratory was AOAC Chap.10/GC-MS.

In 2003, the cola companies were supported by the pesticide industry. They claim that malathion gets degraded quickly and therefore they should not be present. This time round CSE has double checked malathion findings and confirmed it through a state-of-the-art GC-MS equipment - Finnigan Polaris Q Ion trap GC/MSⁿ with EI ionization (70eV), in Selective Ion Monitoring (SIM) mode by evaluation of the mass spectral pattern of the sample and library match within NIST/Main/Pesticide spectral library. The SIM mode spectrum of malathion of the sample with the primary ion located at m/z 93 and secondary ions at m/z 79, 125, 127, 55, 99, 143, 158, 173, matched with the library search in first hit and 94% probability. The GC-MS spectra, therefore, unequivocally detects and confirms malathion. This is the most credible, and scientifically advanced, evidence to show the presence of malathion in soft drink samples.

5 Expert Committee: Heptachlor is banned from 1996. Hence, its presence in such concentration is unlikely. GC-MS chromatogram also confirms absence of heptachlor.

CSE response: Heptachlor was banned precisely because it is a highly persistent pesticide. Once used even in small quantities heptachlor will surely stay in the environment for more than 20 years. Therefore not only will heptachlor be found today, it will, in all probability, continue to be found for the next 10 years. The very scientists, in the ministry's expert group, who are questioning heptachlor findings, have themselves found this pesticide in different kinds of samples from all over the country.

The finding of Heptachlor in soft drinks is not the first instance of Heptachlor detection in India after it was banned in 1996. The tests done by the Indian Council of Agricultural Research (ICAR) of the Government of India itself has reported presence of Heptachlor in many food commodities in 2001-2002. So have many other researchers and academic institutions of the country. In two of the most recent studies done by the government institutions, Heptachlor was detected in water samples, soil samples, processed food commodities and vegetables.

- Monitoring of pesticide residues in ground water (open well and tube well) and surface water (canal, lake and ponds) was carried out by CCS Haryana Agricultural University (CCSHAU), Hisar in 2000-2001. CCSHAU is a pesticide research centres under the All India Coordinated Research Project on Pesticide Residues of Indian Council of Agricultural Research. In its tests it found Heptachlor in water samples from Hisar. This same centre also found Heptachlor in soil samples from different cropping systems. It also found Heptachlor in processed food commodities like honey, jam, jellies and sauce.
- A study conducted by the Chandigarh based Post Graduate Institute of Medical Education and Research (PGIMER) in 2004 on pesticide residues in food commodities and water in Bhatinda district of Punjab found Heptachlor in both food commodities and water in high quantities. Heptachlor was found in both ground water and tap water. In vegetable samples also Heptachlor were found.

Ministry's expert groups assertion is not correct when they say that CSE report does not confirms presence of heptachlor. First of all, Heptachlor presence was identified and quantified by GC-ECD on the basis of retention time and peak area with reference to the standard. It was further confirmed by evaluation of mass spectral pattern of the sample and comparison with library match results within NIST/Main/Pesticide spectral library. Heptachlor was confirmed by primary ion at m/z 65 and supported by four secondary ions at 100, 103, 66 and 337.

6. Expert Committee: Chlorpyrifos: The mass fragmentation pattern of standard (Rt 19.36) and sample (Rt 18.54') points towards presence of chlorpyrifos residues in the sample. However, Rt of both do not match.

CSE response: The qualitative identification of any compound is based on comparison of the sample mass spectrum, with characteristic ions in a reference mass spectrum. The primary ion (m/z) should be supported by two secondary ions between the reference and the sample spectrum.

The characteristic ions from the reference mass spectrum are defined as three ions of greatest relative intensity or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. {Reference: EPA Method 8270 C- Semi volatile organic compounds by Gas Chromatography /Mass Spectrometry (GC/MS)}. Presence of chlorpyrifos was confirmed by primary ion at m/z 97 and supported by six secondary ions

at 125, 197, 199, 258, 286, 314 and 316 in the GC MS spectra of the sample in first hit and with 95% probability.

7. Expert Committee: The review of literature is not based on the balanced approach.

CSE response: The literature review in CSE's report talks about issues other than those pertaining to the test report. In our review, we have talked about constituents of soft drinks such as caffeine, acids and other additives. All these constituents are harmful.

Scientific studies are empirical and do not leave any scope for balance or unbalance. We have presented the already available scientific research. Thus, it would seem that the expert committee's criticism itself is unbalanced.

Reply to additional points raised by the expert committee

About the Analytical methodology: The expert committee has stated that the USEPA methods mentioned in CSE report is not for the quantitative estimation of pesticides in soft drinks in particular. These methods are meant for estimation of pesticides in solids and liquids.

CSE Reply: This statement of the expert group clearly exemplifies the biased nature of their examination of CSE report. Are soft drinks not liquid? USEPA methodology is a generic methodology for detecting pesticides in solid and liquid samples. If one starts looking at specific methodology for each and every product in the market, then no one can test any product. This is the standard objection raised by the cola companies when one talks about setting standards for soft drinks and it seems that the expert group is favourably inclined towards their opinion.

But before making such a statement, the expert group should have looked at the JPC report and found that all the tests done on soft drinks in India so far have used the USEPA methodology only. The tests done by the health ministry own labs – CFL- Calcutta and CFT-Mysore – used USEPA methodology.