

A N A L Y S I S O F

**PESTICIDE  
RESIDUES**

I N S O F T D R I N K S

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## 1. ABOUT THE CSE LABORATORY

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The Centre for Science and Environment (CSE), a non-governmental organisation based in New Delhi, has set up the Pollution Monitoring Laboratory (PML) to monitor environmental pollution. Its main aim is to undertake scientific studies to generate public awareness about food, water and air contamination. It is equipped with state-of-art equipments for monitoring and analysis of air, water and food contamination, including High Performance Liquid Chromatograph (HPLC), Gas Chromatograph (GC) with ECD, NPD, FID and other detectors, UV-VIS Spectrophotometer, Mercury Analyzer, Respirable Dust Sampler etc. It provides scientific services at nominal cost to communities that cannot obtain scientific evidence against polluters in their area. Given the state of scientific research in India --most of it being restricted to national defense and food security -- this is an effort to use science to achieve ecological security.

## 2. INTRODUCTION & ORIGIN OF THE STUDY

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After the publication of the PML study on pesticides residues in bottled water which reported the presence of multiple pesticide residues in almost all the brands, a spate of letters were received enquiring about the presence of pesticide residues in soft drinks. In response to this public query it was decided to conduct a similar study in soft drinks.

A total of 12 brands-most popular and easily available -- were considered in this particular study. PML tested the samples with a widely and internationally used methodology based on United States Environment Protection Agency (USEPA) methodology for organochlorine pesticide and organophosphorus pesticide detection. Extraction was done as per the given methodology and analysis by Gas Chromatograph with Electron Capture Detector and with Nitrogen Phosphorus detector using a capillary column.

## 3. SOFT DRINK INDUSTRY AND REGULATIONS

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### 3.1 Definition of soft drink

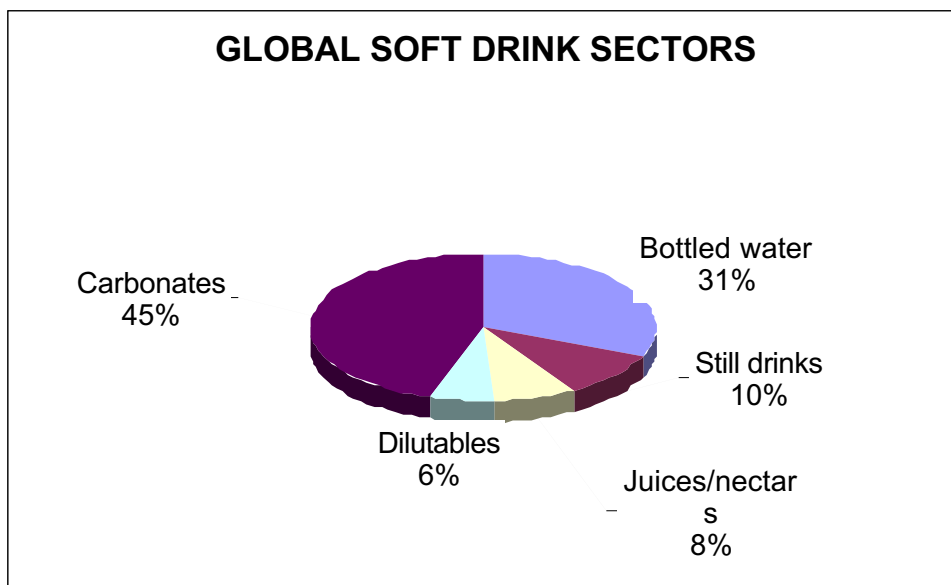
Soft drinks are non-alcoholic water-based flavored drinks that are optionally sweetened, acidulated, carbonated and which may contain fruit, fruit juice and/or salts; their flavor may derive from vegetable extracts or other aromatic substances. They constitute a defined and homogenous range, designated by a generic denomination and utilizing a single common list of additives. They include the beverages which comply with this definition, which utilize these additives and which do not claim to be part of adjacent categories such as fruit juices and nectars, dairy drinks, mineral waters, etc.

### 3.2. The Market

#### ***Global Scenario***

Globally, carbonated soft drinks are third most consumed beverages. Per capita annual consumption of carbonated soft drinks is nearly four times the per capita consumption of fruit beverages. (Source: Data from the Beverage marketing Corporation, as reported by the Canadian Soft drink Association). Soft drink consumption is growing by around 5% a year, according to Global Soft drinks 2002. (Zenith International, 2002). Total volume reached 412,000 million litres in 2001, giving a global per capita consumption of around 67.5 litres per year.

## GLOBAL SOFT DRINKS CONSUMPTION, 2001



Source: Zenith International, 2002 [www.globaldrinks.com](http://www.globaldrinks.com)  
viewed on BevNET.com ([www.bevnet.com/news/2002/11.1.2002-Zenith.asp](http://www.bevnet.com/news/2002/11.1.2002-Zenith.asp))

North America is the largest soft drinks market with 27 per cent of total world soft drink sales and a consumption of 48 gallons per person per year (192 litres/ person / year). The European market accounts for 21 per cent, with a per capita consumption of 12.7 gallons per year (50.8 litres / person/ year). The fastest growth in soft drink consumption is in Asia and South America ([www.beveragemarketing.com/news2p.htm](http://www.beveragemarketing.com/news2p.htm)). Carbonated drinks are the biggest soft drinks sector with 45% of global volume. The five fastest growing soft drink markets between 1996 and 2001 were from Asia, East Europe and the Middle East. The five fastest developing markets during 2001 and 2006 are all expected to come from Asia. Amongst them Pakistan is predicted to have the highest percentage growth rate while India is expected to make sizeable volume gains, as affluence spreads to more of its vast population. Indonesia, China and Vietnam complete the top five for future growth. The overall market should hit 523,000 million litres by 2006. A continuing 5% growth rate for soft drinks compares favourably with at best 1% for hot drinks, 2% for milk and 3% for alcohol. (Source: Zenith International, 2002).

#### ***Major Players-Global***

The global soft drink industry is highly concentrated, being largely controlled by multinational companies. Among the major players are Coca-Cola Co. and PepsiCo. Coca-Cola leads the carbonated drink market in most countries in the world with 60% of the global cola market with its flagship Coca-Cola brand. Other notable players include Cadbury Schweppes.

#### ***Indian Scenario***

##### ***Market***

According to government estimates soft drinks marketed in India were 6540 million bottles in March 2001. The market growth rate, which was around 2-3% in '80s, increased to 5-6% in the early '90s and is presently 7-8% per annum. Most of the sales of soft drinks takes place during summers while just 5-6% of total sales take place in winters. In summers the high season lasts for 70-75 days, which contributes more than 50% of the total yearly sales. In terms of regional distribution cola drinks have main markets in metro cities and northern states of UP, Punjab, Haryana etc. Orange flavored drinks and sodas are popular in southern states. Western markets have preference towards mango-flavored drinks.

##### ***Major Players in India***

The two global majors PepsiCo and Coca-Cola Co. dominate the soft drink market in India. Coca-Cola, which had wined up its India operations during the introduction of the FERA regime, re-entered India 16 years later in 1993. Coca-Cola bought local brands-Thumps Up, Limca and Gold Spot from Parle Beverages and soft drink brands Crush, Canada Dry and Sport Cola from Cadbury Schweppes in early 1999. Pepsi started a couple of years before Coca Cola in 1991 has bought over Mumbai based Duke's range of soft drink brands.

There are conflicting figures about their market share. According to one estimate (ORG)- Coca-Cola had 57% of soft drink market and Pepsi had 41 %. While another estimate (IMRB) figures were Pepsi- 49% and Coca- Cola- 48% during the same period January to May 2000. The soft drinks segment, dominated by MNCs, accounted for Rs 6,247 crore in sales in 2002. Thums Up, the brand taken over by Coca-Cola, is estimated at Rs 1,350 crore, bigger than Coca-Cola itself in India. Thums Up and Limca, two key brands that Coke acquired from Parle, account for no less than Rs 1,950 crore in sales for Coke. The Coca Cola Company in India has Rs 3,757 crore of sales of soft drinks, while PepsiCo's three main brands have sales of Rs 2,490 crore. (Source: Economic Times presentation, Retail Biz, 25<sup>th</sup> March 2003)

### **Consumption**

According to NCAER survey 91% of the total consumption of soft drinks in the country is by lower, lower middle and upper middle class people. Per capita consumption in India is among the lowest in the world at 6 bottles per annum compared to 80 bottles in Thailand and 800 bottles in USA. Delhi market has highest per capita consumption in the country with 50 bottles per annum. (India Info line Sector Report, 2002).

### **Types**

Non-alcoholic soft drink beverage market can be divided into fruit drinks and soft drinks. Soft drinks available in glass bottles, aluminum cans, PET bottles or disposable containers can be divided into carbonated and non-carbonated drinks. Cola, lemon and oranges are carbonated drinks and non-carbonated drinks include mango drinks. Soft drinks can also be divided into cola products and non-cola products. Cola products like Pepsi, Coca-Cola, Thumps Up, and Diet Coke, Diet Pepsi etc. account for nearly 61-62% of the total soft drinks market. Non-Cola products constitutes 36%, and based on the types of flavors available can be divided into Orange, Cloudy Lime, Clear Lime and Mango. (India Infoline Sector Report, 2002).

### **3.3 Soft drinks ingredients**

The major ingredients of soft drinks include the following:

#### **Water**

The major ingredient of soft drinks is **water** and it accounts for 86%-90% of the soft drink composition.

#### **Aromatic substances**

Aromatic substances are added to soft drinks to give a pleasant taste and better stability to the taste. These could be natural aromatic substances like **caffeine** obtainable from a variety of leaves, seeds and fruits. Identical aromatic substance can be obtained more simply and cheaply, in purer forms from raw materials other than plant raw materials and have characteristics which correspond exactly with their natural equivalents.

#### **Sweeteners**

There are many different types of sweeteners like **sugar** (sacharose), another major ingredient in soft drinks as it is highly nutritious and is the invaluable carrier of the fruit aromas. It is made from sugar-beet or sugar-cane or sweeteners found naturally in many fruits and vegetables. Two simple types of sugar are found in fruits - fructose (fruit-sugar) and glucose (grape-sugar). There are also low-calorie artificial sweeteners like saccharin and aspartame (nutra-sweet). **Saccharin**, is a non-nutritious sweetener which is extremely sweet, stable gives no energy (no calories). **Aspartame** is a nutrient-sweetener built up of two amino-acids, asparagin acid and phenylalanine (200 times sweeter than saccharin).

#### **Carbon dioxide**

**Carbon dioxide** is another important ingredient added to the soft-drinks in liquid form. It makes the drink more refreshing through its stimulation of the mouth's mucous membranes adding a sensation that the soft drink is colder than it actually is. The carbon dioxide also brings out the aroma since the carbon dioxide bubbles 'drag with them' the aromatic components. It also checks microbiological growth.

#### **Acids**

The most common acids used in soft drinks are **citric acid**, **phosphoric acid** and **malic acid**. The function of acidity in the drink is to balance the sweetness, make the drink fresh and thirst-quenching.

### **Colouring matter**

Colour is added to soft drinks to make them presentable and appetizing. Brown drinks are colored with **caramel** (when sugar is heated, its colour changes to brown, it becomes less sweet and acquires a burnt taste) or betakarotin, which is also the dominant colouring agent in carrots and oranges.

### **Preservatives**

Preservatives like **sodium benzoate** and **potassium sorbate** are added to increase the life of the product. Sulphur dioxide can also be used as a preservative.

### **Antioxidants**

Antioxidants are substances, which prevent reactions that destroy aromatic substances in soft drinks. The most common antioxidant used is **ascorbic acid**, i.e. Vitamin C

### **Other additives**

Emulsifying agents, stabilizing agents, and thickening agents are also added so that the contents of the drinks remain evenly distributed. Examples of stabilizing agents and thickening agents are **pectin**, which is obtained from citrus fruits or apples, and **alginates** and **carraghen**, which is obtained from algae. ([www.unesda-cisda.org/public/framehealth.htm](http://www.unesda-cisda.org/public/framehealth.htm))

## **3.4 Manufacturing process**

The production of soft drinks begins by making a syrup of sugar and water and an aromatic mixture (soft drink extract) made of raw fruit-juice, other aromatic agents as well as an acid. Soft drinks are acidified either by the addition of fruit juice or by the inclusion of an acid such as that found naturally in fruits (malic or citric acid) or phosphoric acid which is generally used in cola drinks. The components are then mixed into a soft drink concentrate - a syrup.

The water used is treated to remove the oxygen to avoid reactions which destroy the aromatic substances (oxidation). In case of carbonated drinks, the water is 'carbonated' with carbon dioxide under high pressure. All the air is removed to prevent froth formation when the package is opened. The syrup and the carbonated water are mixed in the correct proportions. The drink is then bottled, canned or put into other packaging for retail sale. ([www.unesda-cisda.org/public/framehealth.htm](http://www.unesda-cisda.org/public/framehealth.htm))

## **3.5. Regulations**

In **India** the soft drinks Industry is virtually unregulated. Rule 65 of the Prevention of Food Adulteration Act 1954, regulates the presence of insecticides and pesticides in food but "food" is so defined in Rule 65 as to exclude "beverages". This Rule does not apply to soft drinks. Subsection A.01.01 in appendix B of PFA defines standards of quality for non-alcoholic beverages but makes no mention of pesticide residues.

There are specifications for "sweetened aerated water with no fruit juice or pulp or containing less than 10% fruit juice or fruit pulp" in part II (D) of the Fruits Products Order (FPO), 1955. It regulates the general characteristics of a beverage. On the quality of basic raw material-water it merely says "water used in the manufacture shall be potable and if required by the licensing officer shall be got examined chemically and bacteriologically by any recognized laboratory". The order however does not define what is potable nor does it provide any scope to regulate pesticide residues.

The Bureau of Indian Standards (BIS) has laid down specifications IS :2346:1992 for "carbonated beverages". In the foreword to this document water is mentioned as ingredient in carbonated beverages "the quality of carbonated beverage depends on the quality of *various ingredients* that goes into its manufacture -

water, acidulants, sweetening agents, emulsifiers, and stabilizers, flavor, color and carbon dioxide being the important ones”(emphasis added). The document then prescribes the requirements and methods by which the quality of carbonated beverages may be gauged. As part of the process, it lists the various ingredients that can be used to make carbonated beverages. In this list there is no mention of water. This BIS standard is voluntary in nature (unlike mandatory certification for bottled water).

The BIS has another standard- IS :4251-1967 (reaffirmed 1977) which prescribes standards for “Quality tolerances for water for processed food Industry”. In its foreword it says: “In processed food Industry, water is used for a number of purposes such as processing, washing, flushing and general usage and also for boiler feed and cooling”. It does not mention its use in making soft drinks. In any case it does not mention anything about pesticide residues.

The soft drink industry remains not only unregulated but it is also exempted from the provisions of Industrial licensing under the Industries (Development and Regulations) Act, 1951. It gets a one time license to operate from the ministry of food processing Industries, which includes a no objection certificate from the local government and a water analysis report from a public health laboratory. It also requires a no objection certificate from the concerned State Pollution Control Board. There is no mandatory requirement for Environmental Impact Assessment or siting regulations for the Industry. Its use of water- largely unpriced ground water is not regulated.

In contrast in **United States**, regulations provide that the standard of water used to make soft drinks must be the same as that used to make bottled water. “Raw water “ used to make bottled water falls under the purview of US Food and Drug Administration (FDA). According to the rules water consumed in this form is a “ food” therefore water used as an ingredient in beverages must meet the same standards as bottled water.

In addition to the US Safe Drinking Water Act a federal legislation under the Environmental Protection Agency (**EPA**) stipulates drinking water standards to protect public health. Its primary standards are legally enforceable nation wide. In the state of Massachusetts regulations stipulate that the source water used for bottled water and carbonated drinks must meet the Federal EPA National Primary Drinking water standards.

In **Europe**, the European Economic Council Directive 80/778/EEC and 98/83/EEC lay down the standards for quality of drinking water intended for human consumption. Such water it clearly specifies, shall include water used in any food production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption, or effecting the wholesomeness of the food stuff in its finished form”.

#### 4. STANDARD FOLLOWED

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Different International Agencies -- World Health Organization (WHO), Food and Agriculture Organization (FAO), the US Environment Protection Agency (USEPA)/ Food and Drug Administration Act(FDA) stipulate different limits for different pesticides. The European Economic Community’s Directive (80/778/EEC) on “quality of water intended for human consumption” regulated at European level sets maximum admissible concentration for individual pesticides and related products in drinking water at 0.1 µg/L (0.0001mg/L) to ensure that the concentration is low enough to be toxic to human beings. This Directive shall be replaced and repealed by Directive 98/83/EEC on “quality of water intended for human consumption” with effect from December 25, 2003. The limit for pesticides is the same as in Directive 80/778/EEC except for aldrin, dieldrin and heptachlor and heptachlor epoxide for which the parametric value has been made even more stringent at 0.030 µg/L (0.00003 mg / L).



In the present work, PML used European norms to compare the results of pesticide residue analysis in soft-drinks as norms for multiple residues do not exist in most regulations. These norms – for single and multiple residues -- have been recently gazetted by the Ministry of Health and will be applicable to the packaged drinking water in India.

## 5. LITERATURE REVIEW

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The major ingredients found in soft drinks like water, aromatic substances, sweeteners carbon dioxide, coloring matter, acids, preservatives, antioxidants and other additives are present in different composition in different soft drinks. There is a growing concern in the medical and scientific communities about the harmful effects associated with the consumption of carbonated soft drinks.

Studies have shown that soft drinks can lead to numerous health problems. Some of these health problems are obesity, diabetes, tooth decay, osteoporosis, nutritional deficiencies, heart disease, and many neurological disorders. Various ingredients used in soft drinks can have adverse effects.

A common problem that is associated with consumption of a large number of soft drinks is the increased acid levels (citric, malic and phosphoric acid) throughout the body causing gastronomic distress due to the inflammation of the stomach and erosion of the stomach lining leading to painful stomachache as the stomach which maintains a very delicate acid-alkaline balance can be set out of balance by the consumption to a large number of soft drinks, which can create a constant acid state leading to indigestion and gassiness.

Carbon dioxide emitted from soft drinks is a waste product that humans excrete and can be harmful when ingested. Large amounts of sugar, bubbles caused by carbon dioxide, and phosphoric acid that are found in soft drinks remove nutritious minerals from bones allowing the bones to become weak and increasing the risk for them to break. This is done by the phosphoric acid disrupting the calcium-phosphorous ratio, which dissolves calcium from the bones (Source: <http://www.kauhawaii.com/softdrinks.html>).

It has been reported that enamel, which is composed of cementum and dentin, naturally protects teeth. Acidic drinks increase dentin permeability by opening dentinal tubules leaving a dentin surface completely uncovered and removing the smear layer. (Prati C *et al*, 2003) . Dental cavities are often associated with consumption of carbonated beverage because the amount of sugars that are consumed is important in forming caries caused by the bacteria *mutans streptococi*, which is a part of dental plaque. *Lactobacillus* and *Actinomyces viscosus* are two other kinds of bacteria that adversely affect teeth and survive well in very acidic environments, produce high amounts of acid from sugars and other types of acid.

Comparison of the effects of aspartame-sweetened and sucrose sweetened soft drinks on food intake and appetite ratings of female restrained eaters suggested that substitution of sucrose-sweetened drinks for diet drinks does not reduce energy intake and may even result in higher intake during the subsequent day. (Lavin J H *et al*, 1997)

Caffeine- a methylated xanthine acts as a mild central nervous system stimulant present in carbonated beverages. Large amounts of caffeine consumption can cause diseases and disorders such as insomnia, nervousness, anxiety, irritability, and deviations from the normal heart rate. A major concern about caffeine is that it increases the excretion of calcium in urine, which increases the risk for osteoporosis in heavy caffeine consumers. Some epidemiological studies describe exposure to caffeine during pregnancy as well as the occurrence of congenital malformations, fetal growth retardation, miscarriages (spontaneous abortions), behavioural effects and maternal fertility problems. (Christian WS *et al* , 2001)

Many soft drinks contain caramel which is added to give dark appearance. The caramel coloring used in soft drinks may be a carcinogen. (Source: <http://members.aol.com/profchm/Soft.html>)

Mineral waters and soft drinks were investigated for erosive potential of human teeth and showed that mineral waters offer a safe alternative to more acidic beverages and their complex mineral ion compositions may positively influence any dissolution process at the tooth surface. ( Parry J *et al*, 2001) .

A study was conducted on concentrations of aluminum in drinking waters, fruit juices and soft showed that aluminum ranged from 4.2 to 165.3 µg/l in drinking water, 49.3 to 1,144.6 µg/l in fruit juices and from 44.6 to 1053.3 µg/L in soft drinks. Aluminum in high quantities is known to have adverse effects. (Lopez FF *et al*, 2002)

According to a study conducted on soft drinks, mineral waters and juices available in the Danish market, dissolution of enamel increased logarithmically inversely with the pH of the drink. Orange juice pH 4.0 exhibited high buffering effect, supplemented with 40 mmol/l calcium and 30 mmol/l phosphate did not erode the enamel as the calcium and phosphate saturated the drink with respect to apatite. The capability of a soft drink or a juice to erode dental enamel depends not only on pH of the drink but also on the buffering effect. The latter is the ability of the drink to resist a change in pH and may add to the effect of change in pH. (Larsen MJ *et al*, 1999)

According to Food Safety Information Bulletin, 98, July 1998, Benzene, a hydrocarbon was detected in some carbonated drinks in UK. Investigations revealed that benzene entered into the soft drinks through the contaminated carbon dioxide used for carbonation. Benzene is a genotoxic agent.

## 6. MATERIALS AND METHODS

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### 6.1. Sampling methodology

Soft drink bottles of different brands and flavors were purchased from various markets in Delhi during the month of May 2003. Extraction and pesticide residue analysis was carried out at the PML during the same month. Three samples of each of the 12 different brands (thirty six samples) were analyzed for 16 organochlorines, 12 organophosphorus pesticides and 4 pyrethroides. The 16 organochlorines, which were, tested cover wide spectrum of chlorinated pesticides. The organophosphorus and pyrethroides pesticides were tested are the most commonly used organophosphorus pesticides in India. Details of the samples purchased from India are in Annexure IA and Annexure IB gives the details of the soft drink samples procured from USA and tested in PML.

### 6.2. Equipments

Gas Chromatographs used for pesticide residue analysis were Thermoquest-Trace GC with the <sup>63</sup>Ni selective Electron-Capture Detectors with advanced software (Chromcard-32 bit Ver 1.06 October 98) and Nucon –GC- 5765 series equipped with Nitrogen Phosphorus detector. GC column employed were capillary column, DB-17, J & W make and DB-5, J & W make (for cross verification). Rotatory evaporator (Buchi type) and a 10-µl syringe from Hamilton Co. were employed.

### 6.3. Solvents

All the solvents acetone, methylene chloride, hexane (HPLC) grade used for the analysis were purchased from E-Merck.

### 6.4. Chemicals

Pesticide reference standards were obtained from Sigma Chemicals USA. Weighing appropriate amounts of active ingredients in a 40 ml brown bottle with a Teflon-lined screw cap and dissolving the weighed standard with HPLC grade acetone prepared individual stock solutions. The resulting concentration was corrected for the stated purity. Appropriate aliquots of the obtained solutions were subsequently mixed

into a 50 ml volumetric flask, which was completed to volume with acetone. Anhydrous sodium sulfate, sodium chloride were purchased from s. d. Fine Chem Ltd.

#### **6.5. Sample extraction and Clean up**

The samples were analysed for organochlorines by using EPA method 8081A for organochlorines by Gas chromatography and EPA Method 8141A for organophosphorus compounds by gas chromatography: Capillary column technique.

**Extraction:** Soft drink samples were shaken well and filtered through Whatman filter paper no.1. pH of the samples were checked and it was found to be acidic, pH of all the samples was neutralised by using 0.1 N NaOH. After filtration, 500 ml of sample was taken in a 1 L capacity separatory funnel and 20 ml of saturated sodium chloride solution was added. The water sample was partitioned with 100 ml of methylene chloride (thrice) by shaking the separatory funnel vigorously for 2-3 min releasing the pressure intermittently. The layers were allowed to separate. The three extracts of methylene chloride layers were combined and passed through anhydrous sodium sulfate and concentrated to about 1-2 ml using rotary vacuum evaporator. Again 10 ml methylene chloride was added for adsorption chromatography

**Clean up:** Cleanup was done by EPA Method 3620B- Florisil clean up by column chromatography. Florisil was activated at 130<sup>o</sup> C overnight and cooled in a dessicator before use. Weight of florisil taken was predetermined by calibration using lauric acid. 20g florisil was packed in the 20 mm ID chromatographic column. And anhydrous sodium sulfate was added to the top of the florisil column(1-2 cm) and the column was pre-eluted with hexane. The methylene chloride extract was added to the top of the column and then eluted with Hexane (100ml), 30% methylene chloride in hexane (100ml) and finally with methylene chloride (100ml). Eluent was collected and concentrated to dryness. Final samples were prepared in Hexane (HPLC grade) and analyzed by GC-ECD for organochlorines and synthetic pyrethroides and GC equipped with NPD for organophosphorus pesticides.

#### **6.6. Sample Analysis**

##### *For Organochlorines and synthetic pyrethroides*

Organochlorine and synthetic pyrethroides were analysed by Gas Chromatograph (Thermoquest-Trace GC) with the <sup>63</sup> Ni selective electron-capture detector. This detector allows the detection of contaminants at trace level concentrations in the lower ppb range in the presence of a multitude of compounds extracted from the matrix to which the detector does not respond. The capillary column used was DB- 17- coated with 50% phenyl, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25µm). The carrier gas and the makeup gas was nitrogen with a 0.4 ml/min and 60 ml/min-flow rate respectively employing the split less mode. 2.0µl of the final extract was injected at a temperature of 270<sup>o</sup> C. The oven temperature was kept at 120<sup>o</sup>C with a hold time of 1 minute, then from 120<sup>o</sup>C to 205<sup>o</sup> C at a rate of 25<sup>o</sup> C/minute with a hold time of 1 minute then finally from 205 to 290<sup>o</sup>C at a rate of 2<sup>o</sup> C / minute with a hold time of 12 min. The total run length was 59.9 minutes. The detector was maintained at 320<sup>o</sup>C. Peak identification was performed by the GC software (Chromcard-32 bit Ver 1.06 October 98) calibration table set up with a relative retention time window of 0.65%.

##### *For Organophosphorus*

Organophosphorus pesticides were analysed by Gas Chromatograph (Nucon –5765 series equipped with Nitrogen Phosphorus detector). The capillary column used was another GLC capillary column – DB- 17- coated with 50% phenyl, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25µm). The carrier

gas and the makeup gas was nitrogen with a 1.3 ml/min and 25-ml/min-flow rate respectively, Hydrogen at 8ml/min and Air at 80ml/min respectively employing the split less mode. 2.0µl of the final extract was injected at a temperature of 270<sup>o</sup> C. The oven temperature was kept at 120<sup>o</sup>C with a hold time of 1 minute, then from 120<sup>o</sup>C to 205<sup>o</sup> C at a rate of 25<sup>o</sup> C/minute with a hold time of 1 minute then finally from 205 to 270<sup>o</sup>C at a rate of 2<sup>o</sup> C / minute with a hold time of 1 min. The total run length was 38.90 minutes. The detector was maintained at 300<sup>o</sup>C.

The samples were calibrated (retention time, area count) against standard mixture of known concentration of 12 organophosphorus pesticides. Each peak was characterized by comparing relative retention time with those of standards.

#### 6.7 Calculations

All calculations were done as described in USEPA method and the amount of residues in samples were obtained.

#### Recovery:

Recovery experiment was done as mentioned in extraction methodology, a known amount of standard pesticides was injected in the sample before extraction, to check how much were recovered after complete exercise. Generally with the ten set of extraction one recovery experiment was performed. Recovery was 90% for OCs, 85% for OPs and 88% for synthetic pyrethroides.

The reproducibility of results for all the pesticides was 95 percent and above for all the samples. However, the mean average reading of a individual sample analyzed in triplicate, has been reported in the results.

#### 6.8 Confirmation and Quantification

##### Spiking

Identifications were confirmed by spiking the sample with known standard only to confirm the unknown Thin layer chromatography of the pooled extract was also performed. Solvent systems used were hexane: benzene (4:1, v/v). The spots corresponding to the position of standards were scraped, extracted and analysed by GLC.

##### Dual column

The identifications were crosschecked with another column - DB-5 coated with 5% diphenyl and 95% dimethylpolysiloxane length of different polarity. Elution pattern was different from the elution pattern in DB-17.

### 7 RESULTS AND DISCUSSION

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A total of 36 soft drinks samples of 12 brands (3 each- different batches) were tested for 16 organochlorine pesticides, 12 organophosphorus and 4 pyrethroides pesticides most commonly used in India.

#### **Organochlorines:**

Amongst organochlorines -  $\gamma$ -HCH (Lindane) was detected in 100% of the 36 samples analysed. The range of concentration in the 12 brands varied from 0.0008-0.0042 mg/L. Minimum

concentration was detected in Diet Pepsi- 0.0008 mg/L (8 times the EEC limit) and maximum concentration was detected in Mirinda lemon- 0.0042 mg/L which is 42 times higher than EEC limit for drinking water i.e. 0.0001mg/L limit for individual pesticide as per the Drinking Water Directive 80/778/EEC. **Average concentration of lindane detected in all the samples was 0.0021 mg/L, which is 21 times higher than the EEC limit.** (ANNEXURE:II)

Hexachlorocyclohexane, previously called BHC (benzene hexachloride), is a mixture of eight isomers of which five are found in the crude product ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ). Only  $\gamma$  isomer of HCH was detected in soft drink samples and other isomers of HCH ( $\alpha$ ,  $\beta$  and  $\delta$ ) were within the European norms, which might be because  $\gamma$ -HCH is more resistant to biological and chemical degradation under aerobic conditions (El beit *et al*, 1981) and is most commonly used.  $\gamma$ -HCH (Lindane) appears in the list of pesticides for restricted use (the use of lindane formulations generating smoke for indoor use is prohibited; it can be used for the control of insect pests of field crops) and has powerful insecticidal properties. and is very effective against a wide variety of insects, including domestic insects and mosquitoes.

**DDT along with its metabolites (DDD+DDE)** was detected in 81% of the samples i.e 29 out of 36. The range of concentration in the 12 brands varied from 0.0001 to 0.0042 mg/L. Minimum concentration was detected in Blue-Pepsi. Maximum concentration was detected in Mirinda lemon -- 0.0042 mg/L which is 42 times higher than the maximum EEC limit. **Average concentration of total DDT in all the samples was 0.0015 mg/L, which is 15 times higher than the EEC limit.**

Use of DDT is banned in agriculture. However its restricted use is allowed in public health sector (10,000 MT per annum). DDT was detected in most of the samples perhaps due to its persistent nature. Since DDT is known to undergo metabolic conversion and dehydrochlorination, presence of metabolites of DDT i. e DDD and DDE encountered in this study might be due to such metabolic processes.

Heptachlor (banned with effect from September 20, 1996), aldrin (banned with effect from September 20, 1996), dieldrin (banned with effect from May, 1990), endosulfan, methoxychlor and chlordane were not detected in any of the samples.

#### **Organophosphorus**

Amongst organophosphorus pesticides - **Chlorpyrifos** was detected in 100% of the 36 samples analysed . The range of concentration in the 12 brands varied from 0.0015-0.0072 mg/L. Minimum concentration of 0.0015 mg/L was detected in Sprite, a Coke product and maximum was detected in Mirinda lemon flavor, a Pepsico product, which is 72 times higher than the EEC limit of 0.0001 mg/L for individual pesticides. **Average concentration of 0.0042 mg/L of chlorpyrifos was detected in all the samples that is 42 times higher than the EEC limit.**

Chlorpyrifos is a moderately persistent insecticide effective against mosquito and fly larvae, cabbage root fly, aphids. Chlorpyrifos has become one of the most widely applied insecticides in homes restaurants against cockroaches, termites.

**Malathion** was present in 97% of the 36 samples analysed. The range of concentration in the 12 brands varied from 0.0013 to 0.0196 mg/L. Minimum concentration of 0.0013 mg/L was detected in Sprite and maximum concentration was 0.0196 mg/L which is 196 times higher than the EEC limit. **The average concentration of malathion (0.0087 mg/L) was 87 times higher than the EEC limit.** Malathion was present in all samples except one sample of Sprite (BN 787).

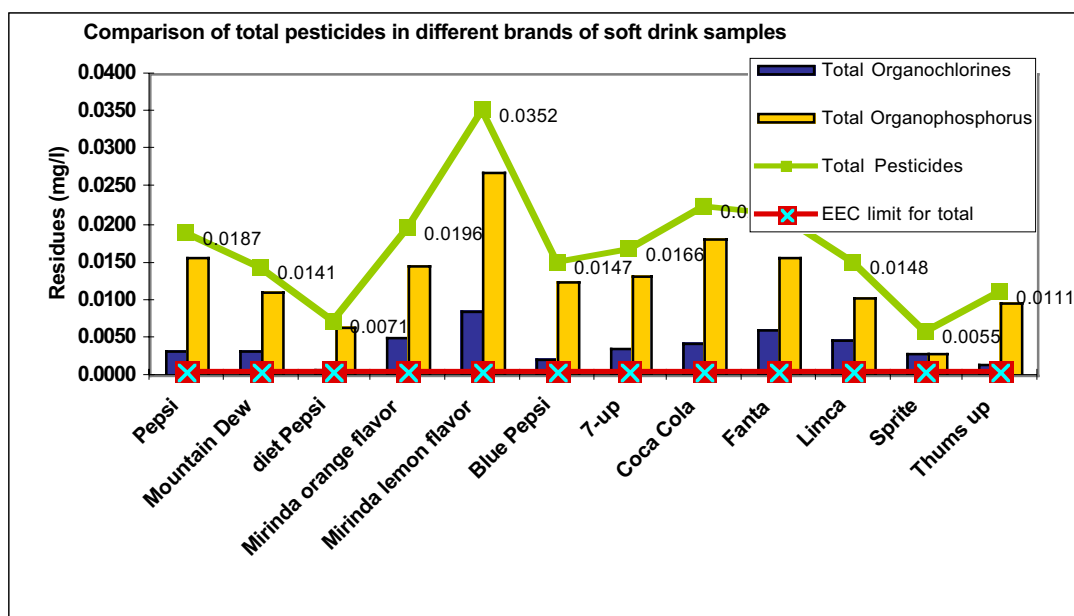
Malathion is an important and widely used contact insecticide and acaricide for the control of aphids, red spider mites, leaf hoppers and thrips on a wide range of vegetable and other crops. It is also

used to control insect vectors like mosquitoes. It is rapidly absorbed by practically all routes including the gastrointestinal tract, skin, mucous membranes, and lungs. Malathion requires conversion to malaoxon to become an active anticholinesterase agent. Most of the occupational evidence indicates a low chronic toxicity for malathion.

The organophosphorus pesticides are less persistent in water, soil, food and feed for animals than the organochlorine pesticides; however they are relatively soluble in water and are highly toxic. They break down into nontoxic metabolites. (ANNEXURE:III)

### Synthetic Pyrethroides

Out of 4 synthetic pyrethroides - permethrin, detamethrin, cypermethrin and fenvalerate, none was detected in any of the soft drink samples.



### Total pesticides residues

The range of concentration of total organochlorines in the 12 brands varied from 0.0008-0.0084 mg/L. Minimum concentration of 0.0008 mg/L was detected in Diet Pepsi and maximum concentration was detected in Mirinda lemon- 0.0084 mg/L. **Average concentration of total organochlorines was 0.0038 mg/L in all the 36 samples.**

The range of concentration of total organophosphorus in the 12 brands varied from 0.0028-0.0268 mg/L. Minimum concentration of 0.0028 mg/L was detected in Sprite and maximum concentration of 0.0268 mg/L was detected in Mirinda Lemon. **Average concentration was 0.0129 mg/L in the 36 samples analysed.**

The range of concentration of total pesticides ( organochlorines and organophosphorus) pesticides in the 12 brands varied from 0.0055-0.0352 mg/L. Minimum value of 0.0055 mg/L was detected in Sprite which is 11 times higher than the EEC limit and maximum value was detected in Mirinda lemon-0.0352 mg/ which is 75 times higher than the total EEC limit of 0.0005 mg/L. **Average concentration of total pesticides detected was 0.0168, which is 34 times higher than the total EEC limit.** (ANNEXURE:IV and Table 2)

The variation in different brands could be due to the different ingredients present in different brands, composition and pH.

**Table: 2 Average residues in different brands of soft drink samples**

S. No.	Brand	Total Organochlorines (mg/L)	Total Organophosphorus ( mg/L)	Total Pesticides (mg/L)	EEC limit for total pesticides -0.0005 mg/L	Deviation from EEC limit (No. of times)
1	Pepsi	0.0032	0.0155	0.0187	0.0005	37
2	Mountain Dew	0.0033	0.0108	0.0141	0.0005	28
3	Diet Pepsi	0.0008	0.0063	0.0071	0.0005	14
4	Mirinda orange	0.0050	0.0146	0.0196	0.0005	39
5	Mirinda lemon	0.0084	0.0268	0.0352	0.0005	<b>70</b>
6	Blue Pepsi	0.0022	0.0125	0.0147	0.0005	29
7	7-up	0.0036	0.0130	0.0166	0.0005	33
8	Coca Cola	0.0044	0.0179	0.0223	0.0005	45
9	Fanta	0.0060	0.0154	0.0214	0.0005	43
10	Limca	0.0047	0.0101	0.0148	0.0005	30
11	Sprite	0.0027	0.0028	0.0055	0.0005	<b>11</b>
12	Thums up	0.0015	0.0096	0.0111	0.0005	22

Note: Average of 3 samples .

Total pesticide residues in the 36 soft drink samples manufactured in India was **0.0168 mg/l** which is **34 times the** EEC limit for total pesticides. Total pesticide residues in all brands of PepsiCo products (India) were 0.0180 mg/L, which is 36 times the total pesticide limit and in all brands of Coca - Cola Co (India) were 0.0150mg/L which is 30 times the total EEC limit for pesticides.

#### **Comparison of Indian soft drink samples with US samples**

The Indian Coca-Cola had pesticide residues 45 times higher than the EEC norms, while no pesticides were detected in the same product procured from the US. Similarly, the Indian Pepsi had pesticide residues 37 times higher than EEC norms while its US counterpart was without any pesticide residues.

### **8. HEALTH IMPACTS OF PESTICIDES**

The four commonly found pesticides detected in the soft drink samples were – Lindane, DDT and its metabolites, Malathion and chlorpyrifos. Health impacts of these pesticides are as follows:

#### **LINDANE**

All isomers of HCH are stored in fats; the gamma isomer of HCH (Lindane) which was also found in soft drink samples analysed is stored at much larger rates than the other isomers, which are more readily metabolized and eliminated. Lindane is absorbed through respiratory, digestive or cutaneous routes and

accumulates in fat tissues. It damages human liver, kidney neural and immune systems and induces birth defects cancer and death. Chronic administration results in endocrine disruption in birds as well as in mammals.(Pages N *et al*, 2002)

Treatment with 1-40 mg of lindane/kg of body weight disrupts testicular morphology, decreases spermatogenesis, inhibits testicular steroidogenesis, reduces plasma androgen concentrations and may adversely affect reproductive performance in males. In females lindane disrupts the estrous cycle, reduces serum estrogen and progesterone levels decreases sexual receptivity

There has been a link of Lindane to immune system. Lindane is a **potent carcinogen**. Rats exposed to gamma HCH showed evidence of liver cancer. (ATSDR, 1989). Chronic exposure to Lindane has been linked to increase in the risk of cancer of the aerodigestive tract and strong **genotoxic** effects on human tonsillar epithelium.(Source: J. Soc Biol, 2002 196(4):339-48)

Lindane was found to be **estrogenic** to female rats and mice, and also caused the testes of male rats to become atrophied. Seminiferous tubules and Leydig cells (important for production of sperms) were completely degenerated at doses of 8 mg/Kg/day over a 10-day period (Gallo MA and Lawryk NJ, 1991).

The absorption of high doses of gamma - HCH is particularly toxic for the central nervous system and for the female and male reproduction apparatus in mammals where lindane is considered as an endocrine disruptor. Lindane is **highly lipophilic** and incorporates into biological membranes according to the following sequence: mitochondria >sarcoplasmic reticulum >myelin >brain microsomes >erythrocytes. Lindane exerts a stimulating action on synaptic transmission and inhibits the chloride current activated by gamma-amino butyric acid (GABA) of many muscular and nervous preparations by interacting with the receptors GABA - chloride channel complex. It seems to affect calcium homeostasis of many tissues. Lindane affects the excitable membranes and the cardio circulatory system. These alterations (may) represent a potential risk for human health. (Sauviat M *et al*, 2002).

#### **DDT and its metabolites**

DDT (dichlorodiphenyltrichloroethane) and its metabolites were detected in 81% of the soft drink samples. They have been linked to altered sexual development in various species, to a decrease semen quality and to increased risk of breast cancer in women. (Sharpe RM, *et al* 1993; Carlsen E *et al*, 1992; Stone R *et al*, 1994). DDT and its metabolites have also been shown to mimic **estrogen**, binding to and activating the estrogen receptors (ER's) thereby often producing estrogen like effects (Jaga K, 2000) They may alter a number of harmful estrogen-regulated health effects in humans such as breast cancer (Cocco P *et al*, 2002) , spontaneous abortion (Korrick SA, *et al*, 2001), reduced bone mineral density (Beard J *et al*, 2000). DDT and its metabolites because of their **lipophilicity** and long half lives accumulate in the food chain. Their weak oestrogenic effects may result from altered metabolism and competition for binding to cytosolic and nuclear receptors of steroid hormones. (Levine R, *et al* 1991)

DDT reportedly induces cancer in animals, mimics estrogen activity, induces antiandrogen effects, and impairs Natural Killer (NK) cells and T lymphocyte responses. Occupational exposure to insecticides resulted in frequent infections and **immunological abnormalities** DDT, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD) in blood levels have been associated with several immune parameters in patients occupationally exposed to insecticides. The majority of 49 patients who worked as farmers or farmhands in the former German Democratic Republic, were contaminated with more than 1 chemical-- most commonly DDE, PCBs, and HCB and 80% of them had been exposed for more than 20 years. (Daniel V *et al*, 2002).

Comparison of blood levels of HCB and total DDT in 159 women with breast cancer and 250 presumably healthy controls showed that mean levels of total DDT and HCB were significantly higher for



breast cancer patients than for controls. No differences in serum levels of total DDT or HCB were found between estrogen receptor positive and estrogen receptor negative patients with **breast cancer** which implies that persistent pollutants may occur in higher concentrations in blood samples from breast cancer patients from controls. (Charlier C *et al*, 2003)

There are mixture effects even when each mixture component is present at concentrations that individually produces insignificant effects. Lifetime treatment of mice with DDT induced liver tumours in a dose related manner and the tumors included overtly metastasizing hepatoblastomas (Hoyer AP *et al*, 1998). Main metabolites of DDT (pp' DDE and pp' DDD) are both **carcinogenic**. Exposure to DDE resulted in high incidence of liver tumors in both male and female mice. The combined exposure to DDE and DDD resulted in a marked increase and early appearance of liver tumors in both sexes (Turosov VS *et al* ; 1973).

Mixture of 4 organochlorines (op' DDT, pp' DDE,  $\beta$ -BHC and pp' DDT) acted together to produce proliferative effects in MCF-7 human breast cancer cells and the combined effect was additive (Gertrudis C *et al* 2001). A study suggests that exposure to a mixture of DDT, HCH and endosulfan and decreased fertility in males, an increase in birth defects and in neonatal deaths (Rupa DS, 1991). Detoxification processes both in humans and animals involve conversion of DDT to less toxic acetate; little is known about variations from person to person in these detoxification mechanisms, and even less about intermediate metabolism concerned. Regardless of detoxification mechanisms, DDT is stored cumulatively in body fat and excretion is extremely slow even after intake ceases. (Smith MI, 1946)

### CHLORPYRIFOS

Chlorpyrifos, one of the most widely used organophosphorus pesticide has been reported to be a developmental neurotoxicant specifically targeting the immature brain. (Barone *et al* 2000; Pope 1999). Fetal and childhood exposure to chlorpyrifos has raised concerns about developmental neurotoxicity. Exposure to chlorpyrifos resulted in adverse effects on brain cell development and cholinergic biomarkers. Neonatal rats were found to be more sensitive to chlorpyrifos than the fetal rats and animals exposed prenatally developed behavioral deficits in adolescence and adulthood. (Qio D *et al*, 2003). Developmental neurotoxicity of chlorpyrifos is thought to involve both neurons and glia, increasing the vulnerability of the developing brain. The vulnerability increases from the gestational exposure through later periods of development which glial neuronal interactions influence brain architectural, circuitary and function. Exposures occurring during childhood are as important as those occurring prenatally.

Chlorpyrifos is a suspected **neuroteratogen**. Recent findings suggest that chlorpyrifos has a shifting cellular target, initially impairing the development of neurons and subsequently affecting the glia, which develop much later.(Garcia *et al* 2001, 2002 ). It was evaluated for potential developmental toxicity in rats and was found to show **fetotoxic and teratogenic effects** at maternal dose of 25 mg/kg per day, a dose that also produced maternal toxicity. Fetal weight and viability were decreased and fetal death and early resorption increased at this dose. (Farag AT *et al*, 2003). Studies carried out to evaluate potential toxicological effects of chlorpyrifos in rats showed that repeated exposure to subthreshold doses of chlorpyrifos may lead to growth retardation, behavioural abnormalities and muscle weakness. (Tery AV Jr. *et al*, 2003)

Chronic exposure to chlorpyrifos has been shown to cause **immunological change**. Comparison of chronic health complaints of twenty-nine individuals exposed to chlorpyrifos with respect to peripheral lymphocyte phenotypes; autoantibodies (nucleic acids and nucleoproteins, parietal cell, brush border, mitochondria, smooth muscle, thyroid gland, and central nervous system/peripheral nervous system myelin); mitogenesis to phytohemagglutinin and concanavillin and compared with 3 control groups (i.e., 1 positive 2 negative) showed an increase in CD 26 expression, a decrease in percentage of CD5 phenotype, decreased mitogenesis in response to phytohemagglutinin and concanavillin, and an increased frequency of

autoantibodies. The alterations in these peripheral blood markers were unaffected by medication, age, sex, or season. (Thrasher JD *et al*, 2002)

Chlorpyrifos toxicity becomes acute if transformed to **chlorpyrifos oxon**, which is a potent anticholine esterase, 1000 times more toxic than chlorpyrifos. Active chlorine dispersed in water causes rapid abiotic transformation of chlorpyrifos to chlorpyrifos oxon. Chlorination is commonly used for treatment of domestic water supplies which is a new concern about the safety of domestic use of chlorpyrifos products. (Wu J *et al*, 2003. The effects of chlorpyrifos and its major metabolite chlorpyrifos oxon have been studied in two in vitro models, neuronotypic and gliotypic C6 cells. Chlorpyrifos inhibited DNA synthesis in both cell lines, but had greater effect on gliotypic cells. Chlorpyrifos oxon, the active metabolite that **inhibits cholinesterase**, also decreased DNA synthesis in PC-12 and C-6 cells with a preferential effect on the latter. Diazinon also inhibits DNA synthesis with preference towards C-6 cells but is less effective than chlorpyrifos (Qiao D *et al*, 2001).

### **MALATHION**

Malathion, a known **cholinesterase inhibitor**, responsible for the hydrolysis of body choline esters, including acetylcholine at cholinergic receptors. Primary site of action in insects is nervous system. It was found to **induce progression of malignant transformation** in epithelial cells in the rat mammary glands. It has been shown to induce changes in the epithelium of rat mammary glands, influencing the process of **carcinogenesis**; such alterations occur at the level of nervous system by increasing the cholinergic stimulation (Vladimir T *et al*, 2002). Malathion induces alterations in actin cytoskeleton and in cell adhesion of cultured breast carcinoma cells. (Cabello G *et al* 2003). It has been reported to induce a slight increase in the incidence of **chromosomal aberrations in bone marrow cells** of rats exposed in vivo. (Kawachi T *et al*, 1980). Malathion caused a significant increase in sister chromatid exchange in human foetal lung fibroblasts after a single dose of 40 µg/l or a double dose of 20 µg/l. (Nicholas AH *et al*, 1979). (Cabello G,2003).

In contrast to potent carcinogens, which induce mammary carcinomas in 100 per cent of intact females, parathion and malathion induced 14.3 and 24.3 per cent of mammary carcinomas. Type of tumors induced had papillary adenomatous patterns and ductal carcinomas with cribriform pattern (Willings SR *et al*, 1975). Malathion incorporated through epithelium of skin, mouth and respiratory tract, are activated in the liver by enzymatic processes producing malaaxon. (Silman I, and Futerman A., 1987). It also has been shown to cause **birth defects** in a variety of wildlife and at levels lower than other pesticides. When administered to adult animals, malathion and related thiophosphonates stimulate, and subsequently inhibit, the nicotinic sites in skeletal muscle, resulting in muscle weakness and paralysis. Neonates (newborn babies) are far more sensitive to these agents than adults, mainly because of a slower rate of detoxification of the metabolite (the metabolite in this case would be the liver breakdown product of malathion – malaaxon which has been shown to be far more toxic than malathion itself. (Source: Teratology, 36:7-9, 1987)

It was found to cause DNA abnormalities at all doses (0.02, 0.2, 2 and 20 µg/ml when added to human blood cells drawn from three healthy non-smoking men, aged 23, 24 and 25. It causes a dose-dependent increase in chromosomal aberrations as well as sister chromatid exchanges in human leukocyte cultures. A dose dependent decrease in mitotic index was also observed which suggests that malathion is a **mild mutagen** and at higher concentrations it might cause **genotoxicity** in humans. (Source: Mutation Research, 301:13-17, 1993).

## 9. CONCLUSIONS

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From analysis of 36 samples of 12 different brands of soft drink samples it can be concluded that:

- Out of the 16 organochlorines, 12 organophosphorus and 4 synthetic pyrethroides analysed soft drink samples, Lindane, DDT and its metabolites, Malathion and Chlorpyrifos were most commonly found in 36 soft drink samples tested.
- Lindane ( $\gamma$ -Hexachlorocyclohexane), a potent carcinogen was detected in 100% of the samples analysed. The average concentration detected in all the samples was 0.0021 mg/L, which is 21 times higher than the EEC limit for individual pesticides. Lindane is the most toxic of all the isomers of HCH and has powerful insecticidal properties and is used for the control of insects of field crops and pests in houses.
- DDT (dichlorodiphenyltrichloroethane) was detected in 81 % of the samples analysed. The average concentration of total DDT (DDT+DDD+DDE) in all the samples was 0.0015 mg/L, which is 15 times higher than the EEC limit.
- Chlorpyrifos, a suspected neuroteratogen was detected in 100% of the 36 samples analysed with an average concentration of 0.0042 mg/L of chlorpyrifos which is 42 times higher than the prescribed EEC limit.
- Malathion was present in 97% of the samples analysed with an average concentration of malathion (0.0087 mg/L) which is 87 times higher than the EEC limit. Malathion was present in all samples except one sample of Sprite (BN 787).
- *Synthetic Pyrethroides* Out of 4 synthetic pesticides-cypermethrin, deltamethrin, fenvalerate and permethrin analysed, none was detected in any of the samples.
- The average concentration of total organochlorines was 0.0038 mg/L, that of total organophosphorus was 0.0129 mg/L and the level of total pesticides detected was 0.0168 mg/L, which is 34 times higher than the total EEC limit. The variation in different brands could be due to the different ingredients present in different brands, composition and pH.
- **No pesticide** residues were detected in the Coca-Cola and Pepsi samples from USA manufactured by the same multinationals.

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## ANNEXURE IA

Details of the soft drink samples purchased from Delhi and analysed for pesticide residues .

S.No.	Name	Place of purchase	Date of Purchase	Manufacturer	Date of manufacture	Expiry date	Batch Number	Other Information
1	Pepsi -1	Mayur Vihar	22/5/03	Varun Beverages Ltd, Plot No. 159, RIICO Industrial Estate, Phase-III, Boranada, Jodhpur- 342001	9/5/03	Best before three months from manufacture.	PN-99	Pepsi is the registered Trademark of Pepsico.Inc.USA.
2	Pepsi -2	Malviya Nagar	12/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	20/4/03	Best before three months from manufacture.	P.03.76.06:06	Pepsi is the registered Trademark of Pepsico.Inc.USA.
3	Pepsi -3	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	2/4/03	Best before three months from manufacture.	P.03.54.01:50	Pepsi is the registered Trademark of Pepsico.Inc.USA.
4	Mountain Dew -1	Meharchand Market	15/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	14/4/03	Best before three months from manufacture.	MO.03.19.10:00	Mountain Dew is the registered Trademark of Pepsico.Inc.USA.
5	Mountain Dew -2	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	13/3/03	Best before three months from manufacture.	MO.03.13.15:09	Pepsi is the registered Trademark of Pepsico.Inc.USA.
6	Mountain Dew-3	Malviya Nagar	12/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	28/4/03	Best before three months from manufacture.	MO.03.26.17:12	Pepsi is the registered Trademark of Pepsico.Inc.USA.
7	Diet Pepsi -1	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	8/5/03	Best before two months from manufacture.	DP.03.11.13:21	Pepsi is the registered Trademark of Pepsico.Inc.USA.
8	Diet Pepsi -2	Malviya Nagar	12/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	19/4/03	Best before two months from manufacture.	DP.03.9.14:25	Pepsi is the registered Trademark of Pepsico.Inc.USA.
9	Diet Pepsi-3	Jor-Bagh Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	8/5/03	Best before two months from manufacture.	DP.03.11.18:21	Pepsi is the registered Trademark of Pepsico.Inc.USA.
10	Mirinda orange flavor -1	Malviya Nagar	12/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	22/4/03	Best before three months from manufacture.	MO.03.24.22:08	Mirinda is the registered Trademark of Pepsico.Inc.USA.
11	Mirinda orange flavor -2	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	2/5/03	Best before three months from manufacture.	MO.03.27.15:22	Mirinda is the registered Trademark of Pepsico.Inc.USA.
12	Mirinda orangeflavor -3	Mayur Vihar	22/5/03	Varun Beverages Ltd, Dautana, Chatta Distt., Mathura - 282401	5/5/03	Best before three months from manufacture.	L3	Mirinda is the registered Trademark of Pepsico.Inc.USA.
13	Mirinda lemon flavor -1	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	30/4/03	Best before three months from manufacture.	ML.03.9.21:21	Mirinda is the registered Trademark of Pepsico.Inc.USA.
14	Mirinda lemon flavor-2	Malviya Nagar	12/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	5/4/03	Best before three months from manufacture.	ML.03.07.15:23	Mirinda is the registered Trademark of Pepsico.Inc.USA.
15	Mirinda lemon flavor-3	Meharchand Market	17/6/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	30/5/03	Best before three months from manufacture.	ML.03.13.17.43	Mirinda is the registered Trademark of Pepsico.Inc.USA.

16	Blue Pepsi-1	Prithvi Raj Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	13/4/03	Best before three months from manufacture.	PB.03.19.13:57	Pepsi is the registered Trademark of Pepsico.Inc.USA.
17	Blue Pepsi-2	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	2/4/03	Best before three months from manufacture.	PB.03.18.21:20	Pepsi is the registered Trademark of Pepsico.Inc.USA.
18	Blue Pepsi-3	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	13/4/03	Best before three months from manufacture.	PB.03.19.21:28	Pepsi is the registered Trademark of Pepsico.Inc.USA.
19	7-up -1	Prithvi Raj Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	15/3/03	Best before three months from manufacture.	S.03.02.19.06	7-up is the registered Trademark of Pepsico.Inc.USA.
20	7-up -2	INA Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	16/3/03	Best before three months from manufacture.	S.03.02.00.43	7 up is the registered Trademark of Pepsico.Inc.USA.
21	7-up 3	Khan Market	14/5/03	Jai Drinks Pvt Ltd,Jawahar Lal Nehru Marg, Jaipur-302018	15/3/03	Best before three months from manufacture.	S.03.02.20:24	7 up is the registered Trademark of Pepsico.Inc.USA.
22	Coca Cola -1	Malviya Nagar	12/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	24/4/03	Best before two months from manufacture.	BN 724	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanta GA 30313, USA
23	Coca Cola -2	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	5/4/03	Best before two months from manufacture.	BN 512	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
24	Coca Cola -3	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	26/4/03	Best before two months from manufacture.	BN 738	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
25	Fanta -1	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	30/4/03	Best before 1.5 months from the date of manufacture.	BN 780	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
26	Fanta -2	JorBagh Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	29/4/03	Best before 1.5 months from the date of manufacture.	BN 776	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
27	Fanta- 3	Malviya Nagar	12/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	8/4/03	Best before 1.5 months from the date of manufacture.	BN 537	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
28	Limca -1	Meharchand Market	15/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	27/4/03	Best before two months from manufacture.	BN 747	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
29	Limca -2	Malviya Nagar	12/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	27/4/03	Best before two months from manufacture.	BN 757	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
30	Limca -3	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	27/4/03	Best before two months from manufacture.	BN 753	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
31	Sprite -1	Mayur Vihar	12/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	30/4/03	Best before two months from manufacture.	BN 787	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA



32	Sprite- 2	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	1/5/03	Best before two months from manufacture.	BN 796	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
33	Sprite -3	Malviya Nagar	12/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	1/5/03	Best before two months from manufacture.	BN 791	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
34	Thums up -1	INA Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	24/4/03	Best before two months from date of manufacture.	BN 720	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
35	Thums up -2	JorBagh Market	14/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	24/4/03	Best before two months from date of manufacture.	BN 727	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA
36	Thums up - 3	INA Market	21/5/03	Hindustan Coca-Cola Beverages Pvt Ltd Tehsil- Hapur District-Ghaziabad UP	7/4/03	Best before two months from date of manufacture.	BN 525	For and behalf of Coac-Cola Company,Coca-Cola Plaza, Atlanata GA 30313, USA

### ANNEXURE IB

Details of the soft drink samples purchased from USA and analysed for pesticide residues.

S.No.	Name	Place of purchase	Date of Purchase	Manufacturer	Date of manufacture	Expiry date	Batch Number	Other Information
1	Coca Cola	USA- Longs Drug Store, 1451 Shattuck Ave Berkeley CA 94709, USA	27/6/2003	Bottled under the authority of the Coca-Cola Company by a member of the CCE bottling group Atlanta, Georgia-30339	-	SEP0103	UYF09305	-
2	Pepsi	USA Longs Drug Store, 1451 Shattuck Ave Berkeley CA 94709, USA	27/6/2003	Manufactured by local bottlers for the Pepsi bottling group Inc. Somers N. Y 10589, under the authority of Pepsico, Inc.	-	JUL2103	17320 A041331	-

Note: 1: All the samples purchased from Delhi were sealed in plastic bottles of 500 ml capacity.

## ANNEXURE II

### Organochlorine pesticide residues in soft drink samples

Sl.No	Brands	Batch No.	Residues (mg/L)											
			$\alpha$ -HCH	$\beta$ -HCH	$\gamma$ -HCH	Average of brands - Lindane	$\delta$ -HCH	DDT	DDE	DDD	Total DDT + Metabolites	Average of brands - DDT+Metabolites	Total Organochlorines	Average of brands - Total Organochlorines
1	Pepsi -1	PN-99	ND	ND	0.0015	0.0015	ND	ND	0.0017	ND	0.0017	0.0016	0.0032	0.0032
2	Pepsi -2	P.03.76.06:06	ND	ND	0.0016		ND	ND	0.0012	ND	0.0012		0.0028	
3	Pepsi -3	P.03.54.01:50	ND	0.0003	0.0014		ND	ND	0.0018	ND	0.0018		0.0035	
4	Mountain Dew -1	MO.03.19.10:00	ND	ND	0.0028	0.0025	ND	ND	0.0010	ND	0.0010	0.0008	0.0038	0.0033
5	Mountain Dew -2	MO.03.13.15:02	ND	0.0001	0.0019		ND	ND	0.0006	ND	0.0006		0.0026	
6	Mountain Dew-3	MO.03.26.17:12	ND	ND	0.0027		ND	ND	0.0008	ND	0.0008		0.0035	
7	Diet Pepsi -1	OP.03.11.18:21	ND	ND	0.0007	0.0008	ND	ND	ND	ND	ND	ND	0.0007	0.0008
8	Diet Pepsi -2	OP.09.9.14:25	ND	ND	0.0009		ND	ND	ND	ND	ND		0.0009	
9	Diet Pepsi-3	OP.03.11.20:21	ND	ND	0.0008		ND	ND	ND	ND	ND		0.0008	
10	Mirinda orange -1	MO.03.24.22:08	ND	ND	0.0030	0.0031	ND	0.0019	ND	ND	0.0019	0.0019	0.0049	0.0050
11	Mirinda orange -2	MO.03.27.15:22	ND	ND	0.0032		ND	0.0020	ND	ND	0.0020		0.0052	
12	Mirinda orange -3	L3	ND	ND	0.0031		ND	0.0018	ND	ND	0.0018		0.0049	
13	Mirinda lemon -1	ML.03.9.21:21	ND	ND	0.0044	0.0042	ND	0.0039	ND	ND	0.0039	0.0042	0.0083	0.0084
14	Mirinda lemon -2	ML.03.07.15:23	ND	ND	0.0036		ND	0.0052	ND	ND	0.0052		0.0088	
15	Mirinda lemon -3	ML.03.13.17.43	ND	ND	0.0046		ND	0.0035	ND	ND	0.0035		0.0081	
16	Blue Pepsi-1	PB.03.19.13:57	ND	ND	0.0010	0.0018	0.001	ND	ND	ND	ND	0.0001	0.0020	0.0022
17	Blue Pepsi-2	PB.03.18.21:20	ND	ND	0.0020		ND	ND	0.0003	ND	0.0003		0.0023	
18	Blue Pepsi-3	PB.03.19.21:28	ND	ND	0.0024		ND	ND	ND	ND	ND		0.0024	
19	7-Up -1	S.03.02.19.06	ND	ND	0.0022	0.0020	ND	ND	0.0012	ND	0.0012	0.0013	0.0034	0.0036
20	7-Up -2	S.03.02.00.43	ND	0.001	0.0013		ND	ND	0.0007	ND	0.0007		0.0030	
21	7-Up 3	S.03.02.20:24	ND	ND	0.0025		ND	0.0006	0.0013	ND	0.0019		0.0044	
22	Coca-Cola -1	BN 724	ND	ND	0.0033	0.0035	ND	ND	0.0001	0.0006	0.0007	0.0009	0.0040	0.0044
23	Coca-Cola -2	BN 512	ND	ND	0.0038		ND	ND	ND	ND	ND		0.0038	
24	Coca-Cola -3	BN 738	ND	ND	0.0034		ND	ND	0.0004	0.0017	0.0021		0.0055	
25	Fanta -1	BN 780	ND	0.0003	0.0013	0.0015	0.002	0.0020	0.0011	ND	0.0031	0.0033	0.0067	0.0060
26	Fanta -2	BN 776	ND	ND	0.0018		ND	0.0034	0.0005	ND	0.0039		0.0057	
27	Fanta- 3	BN 537	ND	0.001	0.0015		ND	0.0026	0.0004	ND	0.0030		0.0055	
28	Limca -1	BN 747	ND	0.0001	0.0016	0.0017	ND	0.0028	ND	ND	0.0028	0.0030	0.0045	0.0047
29	Limca -2	BN 757	ND	ND	0.0019		ND	0.0030	ND	ND	0.0030		0.0049	
30	Limca -3	BN 753	ND	ND	0.0017		ND	0.0031	ND	ND	0.0031		0.0048	
31	Sprite -1	BN 787	ND	0.00074	0.0008	0.0014	ND	ND	0.0013	ND	0.0013	0.0009	0.0028	0.0027
32	Sprite- 2	BN 796	ND	ND	0.0012		ND	ND	0.0014	ND	0.0014		0.0026	
33	Sprite-3	BN 791	ND	0.0003	0.0023		ND	ND	ND	ND	ND		0.0026	
34	Thums Up -1	BN 720	ND	0.00053	0.0011	0.0011	ND	ND	0.0002	ND	0.0002	0.0002	0.0019	0.0015
35	Thums Up -2	BN 727	ND	ND	0.0012		ND	ND	0.0003	ND	0.0003		0.0015	

36	Thums Up - 3	BN 525	ND	ND	0.0009		ND	ND	0.0003	ND	0.0003		0.0012	
	Number of samples in which pesticides identified		0	9	36		2	13	20	2	29		36	
	% of total samples in which pesticide residues identified		0	25	100		6	36	56	6	81		100	
	EEC limit(mg/l)		0.0001	0.0001	0.0001		0.0001	0.0001	0.0001	0.0001	0.0001			
	Average		0	0.0001	0.0021	0.0021	0.0001	0.0010	0.0005	0.0001	0.0015	0.0015	0.0038	0.0038
	Deviation of average value from EEC limit.		Within norms	1	21	21	1	10	5	1	2			
	Minimum Amount		0	0.0000	0.0007	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0007	
	Deviation of minimum value from EEC limit.		Within norms	Within norms	7	8	Within norms	Within norms	Within norms	Within norms	Within norms		1	
	Maximum Amount		0	0.0010	0.0046	0.0042	0.0020	0.0052	0.0018	0.0017	0.0052	0.0042	0.0088	
	Deviation of maximum value from EEC limit.		Within norms			42						42		

Note: 1:Average of triplicate

2: ND -Not detected

3:α- endosulfan, β-endosulfan and endosulfan sulfate, heptachlor,aldrin, chlordane,dieldrin, methoxychlor and synthetic pyrethroides - permethrin cypermethrin,

fenvaterate and deltamethrin were also analysed and were not detected.

### ANNEXURE: III

Organophosphorus pesticide residues in soft drink samples collected from Delhi.

SI.No	Brands	Batch No.	Residues (mg/L)					
			Chlorpyrifos	Average of brands - Chlorpyrifos	Malathion	Average of brands - Malathion	Total organophosphorus	Average of brands - Total Organophosphorous
1	Pepsi -1	PN-99	0.0054	0.0062	0.0123	0.0093	0.0177	0.0155
2	Pepsi -2	P.03.76.06:06	0.0070		0.0072		0.0142	
3	Pepsi -3	P.03.54.01:50	0.0063		0.0083		0.0146	
4	Mountain Dew -1	MO.03.19.10:00	0.0036	0.0038	0.0060	0.0069	0.0096	0.0108
5	Mountain Dew -2	MO.03.13.15:02	0.0016		0.0051		0.0067	
6	Mountain Dew-3	MO.03.26.17:12	0.0063		0.0097		0.0160	
7	Diet Pepsi -1	OP.03.11.18:21	0.0039	0.0032	0.0036	0.0031	0.0075	0.0063
8	Diet Pepsi -2	OP.09.9.14:25	0.0027		0.0020		0.0047	
9	Diet pepsi-3	OP.03.11.20:21	0.0029		0.0037		0.0066	
10	Mirinda orange -1	MO.03.24.22:08	0.0069	0.0055	0.0128	0.0091	0.0197	0.0146
11	Mirinda orange -2	MO.03.27.15:22	0.0046		0.0056		0.0102	
12	Mirinda orange -3	L3	0.0050		0.0090		0.0140	
13	Mirinda lemon -1	ML.03.9.21:21	0.0082	0.0072	0.0208	0.0196	0.0290	0.0268
14	Mirinda lemon -2	ML.03.07.15:23	0.0059		0.0164		0.0223	
15	Mirinda lemon -3	ML.03.13.17.43	0.0076		0.0215		0.0291	
16	Blue Pepsi-1	PB.03.19.13:57	0.0034	0.0050	0.0063	0.0075	0.0097	0.0125
17	Blue Pepsi-2	PB.03.18.21:20	0.0074		0.0090		0.0164	
18	Blue Pepsi-3	PB.03.19.21:28	0.0042		0.0072		0.0114	
19	7-Up -1	S.03.02.19.06	0.0024	0.0025	0.0105	0.0104	0.0129	0.0130
20	7-Up -2	S.03.02.00.43	0.0030		0.0118		0.0148	
21	7-Up 3	S.03.02.20:24	0.0022		0.0090		0.0112	
22	Coca-Cola -1	BN 724	0.0043	0.0042	0.0140	0.0137	0.0183	0.0179
23	Coca-Cola -2	BN 512	0.0045		0.0091		0.0136	
24	Coca-Cola -3	BN 738	0.0038		0.0180		0.0218	
25	Fanta -1	BN 780	0.0066	0.0061	0.0170	0.0093	0.0236	0.0154
26	Fanta -2	BN 776	0.0068		0.0062		0.0130	
27	Fanta -3	BN 537	0.0050		0.0047		0.0097	
28	Limca -1	BN 747	0.0029	0.0030	0.0068	0.0071	0.0097	0.0101
29	Limca -2	BN 757	0.0035		0.0073		0.0108	
30	Limca -3	BN 753	0.0026		0.0072		0.0098	
31	Sprite -1	BN 787	0.0016	0.0015	0.0000	0.0013	0.0016	0.0028
32	Sprite -2	BN 796	0.0010		0.0015		0.0025	
33	Sprite -3	BN 791	0.0020		0.0024		0.0044	
34	Thums Up -1	BN 720	0.0024	0.0024	0.0067	0.0073	0.0091	0.0096
35	Thums Up -2	BN 727	0.0021		0.0071		0.0092	
36	Thums Up -3	BN 525	0.0026		0.0080		0.0106	

	<i>Number of samples in which pesticides identified</i>		36		35			
	<i>% of total samples in which pesticide residues identified</i>		100		97			
	<b>EEC limit(mg/l)</b>		0.0001		0.0001			0.0001
	<b>Average</b>		<b>0.0042</b>	<b>0.0042</b>	<b>0.0087</b>	<b>0.0087</b>		<b>0.0129</b>
	<i>Deviation of average value from EEC limit.</i>		42	42.0000	87			
	<b>Minimum Amount</b>		0.0010	0.0015	0.0000	0.0013		0.0016
	<i>Deviation of minimum value from EEC limit.</i>		10	15	Within norms	13		
	<b>Maximum Amount</b>		0.0082	0.0072	0.0215	0.0196		0.0291
	<i>Deviation of maximum value from EEC limit.</i>			72		196		

Note: 1: Average of triplicate

2: ND -Not detected

3: Dichlorvos, diazinon , monocrotofos, phosphamidon, malaoxon, methyl-parathion, quinalphos, phenthoate, profenofos and ethion were also analysed and were not detected.

### ANNEXURE: IV

pH Value, total organochlorines(OCs),total organophosphorus(OPs) and total pesticides in different brands of the soft drink samples.

Sl.No	Brands	Batch No.	PH	Total OCs	Average of brands - OPs	Total OPs	Residues(mg/L)			Average of Company
							Average of brands - OPs	Total pesticides	Average of brands - Total	
1	Pepsi -1	PN-99	2.32	0.0032	0.0032	0.0177	0.0155	0.0209	0.0187	
2	Pepsi -2	P.03.76.06:06	2.37	0.0028		0.0142		0.0170		
3	Pepsi -3	P.03.54.01:50	2.33	0.0035		0.0146		0.0181		
4	Mountain Dew -1	MO.03.19.10:00	2.82	0.0038	0.0033	0.0096	0.0108	0.0134	0.0141	
5	Mountain Dew -2	MO.03.13.15:02	2.81	0.0026		0.0067		0.0093		
6	Mountain Dew-3	MO.03.26.17:12	2.79	0.0035		0.0160		0.0195		
7	Diet Pepsi -1	OP.03.11.18:21	2.47	0.0007	0.0008	0.0075	0.0063	0.0082	0.0071	
8	Diet Pepsi -2	OP.09.9.14:25	2.50	0.0009		0.0047		0.0056		
9	Diet Pepsi-3	OP.03.11.20:21	2.50	0.0008		0.0066		0.0074		
10	Mirinda orange -1	MO.03.24.22:08	2.49	0.0049	0.0050	0.0197	0.0146	0.0246	0.0196	
11	Mirinda orange -2	MO.03.27.15:22	2.58	0.0052		0.0102		0.0154		
12	Mirinda orange -3	L3	2.58	0.0049		0.0140		0.0189		
13	Mirinda lemon -1	ML.03.9.21:21	2.47	0.0083	0.0084	0.0290	0.0268	0.0373	0.0352	
14	Mirinda lemon -2	ML.03.07.15:23	2.41	0.0088		0.0223		0.0311		
15	Mirinda lemon -3	ML.03.13.17.43	2.38	0.0081		0.0291		0.0372		
16	Blue Pepsi-1	PB.03.19.13:57	2.55	0.0020	0.0022	0.0097	0.0125	0.0117	0.0147	
17	Blue Pepsi-2	PB.03.18.21:20	2.58	0.0023		0.0164		0.0187		
18	Blue Pepsi-3	PB.03.19.21:28	2.62	0.0024		0.0114		0.0138		
19	7-Up -1	S.03.02.19.06	3.15	0.0034	0.0036	0.0129	0.0130	0.0163	0.0166	
20	7-Up -2	S.03.02.00.43	3.20	0.0030		0.0148		0.0178		
21	7-Up -3	S.03.02.20:24	3.10	0.0044		0.0112		0.0156		0.0180 PepsiCo.
22	Coca-Cola -1	BN 724	2.30	0.0040	0.0044	0.0183	0.0179	0.0223	0.0223	
23	Coca-Cola -2	BN 512	2.29	0.0038		0.0136		0.0174		
24	Coca-Cola -3	BN 738	2.39	0.0055		0.0218		0.0273		
25	Fanta -1	BN 780	2.58	0.0067	0.0060	0.0236	0.0154	0.0303	0.0214	
26	Fanta -2	BN 776	2.57	0.0057		0.0130		0.0187		
27	Fanta -3	BN 537	2.53	0.0055		0.0097		0.0152		
28	Limca -1	BN 747	2.65	0.0045	0.0047	0.0097	0.0101	0.0142	0.0148	
29	Limca -2	BN 757	2.63	0.0049		0.0108		0.0157		
30	Limca -3	BN 753	2.55	0.0048		0.0098		0.0146		

31	Sprite -1	BN 787	3.10	0.0028	0.0027	0.0016	0.0028	0.0044	0.0055	
32	Sprite- 2	BN 796	3.00	0.0026		0.0025		0.0051		
33	Sprite -3	BN 791	3.00	0.0026		0.0044		0.0070		
34	Thums Up -1	BN 720	2.38	0.0019	0.0015	0.0091	0.0096	0.0110	0.0111	
35	Thums Up -2	BN 727	2.28	0.0015		0.0092		0.0107		
36	Thums Up -3	BN 525	2.46	0.0012		0.0106		0.0118		0.0150 Coca-Cola Co.
	<b>EEC limit(mg/l)</b>							0.0005		
	<b>Average</b>			0.0038		0.0129		0.0168	0.0168	
	<i>Deviation of average value from EEC limit.</i>									
	<b>Minimum Amount</b>			0.0007	0.0008	0.0016	0.0028	0.0044	0.0055	
	<i>Deviation of minimum value from EEC limit.</i>									
	<b>Maximum Amount</b>			0.0088	0.0084	0.0291	0.0268	0.0373	0.0352	
	<i>Deviation of maximum value from EEC limit.</i>									

Note: 1. Average of triplicate.

## ANNEXURE-V

**Standard Deviations of Organochlorine and Organophosphorus residues in soft drink samples collected from Delhi region**

S. No	Name	Organochlorines									Organophosphorus			Total Pesticides	
		$\alpha$ -HCH	$\beta$ -HCH	$\gamma$ -HCH	$\delta$ -HCH	t-HCH	DDT	DDE	DDD	t-DDT	Total Organochlorines	Chlorpyrifos	Malathion		Total Organophosphorus
1	Pepsi	ND	0.0001+0.0002	0.0015+0.0001	ND	0.0016+0.0001	ND	0.0016+0.0003	ND	0.0016+0.0003	0.0032 + 0.0004	0.0062+0.0008	0.0093+0.0023	0.0155 + 0.0019	0.0187 + 0.0022
2	Mountain Dew	ND	ND	0.0025±0.0005	ND	0.0025±0.0004	ND	0.0008±0.0002	ND	0.0008±0.0002	0.0033 + 0.0006	0.0038±0.0022	0.0069±0.0024	0.0108 ± 0.0048	0.0141 ± 0.0051
3	Diet Pepsi	ND	ND	0.0008+0.0001	ND	0.0008+0.0001	ND	ND	ND	0.0008 + 0.0001	0.0032+0.0006	0.0031+0.0010	0.0063 + 0.0014	0.0071 + 0.0014	
4	Mirinda-orange flavor	ND	ND	0.0031±0.0001	ND	0.0031±0.0001	0.0019±0.0002	ND	ND	0.0019±0.0002	0.0050 ± 0.0002	0.0055±0.0013	0.0091±0.0036	0.0146 ± 0.0048	0.0196 ± 0.0047
5	Mirinda lemon flavor	ND	ND	0.0042±0.0007	ND	0.0042±0.0007	0.0042±0.0008	ND	ND	0.0042±0.0008	0.0084 ± 0.0004	0.0072±0.0012	0.0196±0.0064	0.0268 ± 0.0039	0.0352 ± 0.0037
6	Blue Pepsi	ND	ND	0.0018±0.0007	0.0003±0.0008	0.0021±0.0002	ND	0.0001±0.0001	ND	0.0001±0.0001	0.0022 ± 0.0002	0.0050±0.0021	0.0075±0.0014	0.0125 ± 0.0035	0.0147 ± 0.0035
7	7-Up	ND	0.0003±0.0006	0.0020±0.0006	ND	0.0023±0.0002	0.0002±0.0003	0.0011±0.0003	ND	0.0013±0.0006	0.0036 ± 0.0007	0.0025±0.0004	0.0104±0.0014	0.0130 ± 0.0018	0.0166 ± 0.0011
8	Coca-Cola	ND	ND	0.0035±0.0003	ND	0.0035±0.0003	ND	0.0002±0.0002	0.0008±0.0001	0.0010±0.0016	0.0044 ± 0.0009	0.0042±0.0004	0.0137±0.0044	0.0179 ± 0.0041	0.0223± 0.0050
9	Fanta	ND	0.0004+0.0005	0.0015+0.0003	0.0007+0.0011	0.0026+0.0009	0.0027+0.0007	0.0006+0.0003	ND	0.0033+0.0004	0.006 + 0.0006	0.0061+0.0012	0.0093+0.0067	0.0154 + 0.0073	0.0214 + 0.0083
10	Limca	ND	ND	0.0017±0.0002	ND	0.0017±0.0001	0.0030±0.0002	ND	ND	0.0030±0.0002	0.0047 ± 0.0002	0.0030±0.0005	0.0071±0.0025	0.0101 ± 0.0006	0.0148 ± 0.0007
11	Sprite	ND	0.0003+0.0005	0.0014±0.0008	ND	0.0017±0.0007	ND	0.0009±0.0006	ND	0.0009±0.0006	0.0027 + 0.0001	0.0015±0.0005	0.0013±0.0012	0.0028 ± 0.0014	0.0055 ± 0.0013
12	Thums Up	ND	0.0002±0.0003	0.0010±0.0002	ND	0.0012±0.0003	ND	0.0002±0.0001	ND	0.0002±0.0001	0.0015 ± 0.0004	0.0024±0.0002	0.0073±0.0007	0.0096 ± 0.0008	0.0111 ± 0.0006
	<b>Average ± S.D</b>		<b>0.0001 ± 0.0002</b>	<b>0.0021 ± 0.0010</b>	<b>0.0001 ± 0.0002</b>	<b>0.0023 ± 0.0010</b>	<b>0.0010 ± 0.0015</b>	<b>0.0005 ± 0.0005</b>	<b>0.0001±0.0002</b>	<b>0.0015 ± 0.0013</b>	<b>0.0038 ± 0.0020</b>	<b>0.0042 ± 0.0018</b>	<b>0.0087 ± 0.0047</b>	<b>0.0129 ± 0.0060</b>	<b>0.0168±0.0078</b>

- Note 1: Value means- Residues± Standard deviation(S.D)      2: ND-Not detected      3: Average of three samples.  
 4: Heptachlor, aldrin, chlordane, dieldrin, methoxychlor and synthetic pyrethroids - permethrin, cypermethrin, fenvalerate and deltamethrin and organophosphorus pesticides dichlorvos, diazinon, monocrotopos, phosphamidon, malaoxon, methyl-parathion, quinalphos, phenthoate, profenofos and ethion were also analysed and were not detected.  
 5. t-HCH - ( $\alpha, \beta, \gamma, \delta$  - HCH isomers), t-DDT (DDT+DDD+DDE), t- Endosulfan ( $\alpha + \beta$  + sulfate)



## ANNEXURE-VI

### Pesticide Residues in soft drink samples from USA

S.No	Brand	Batch	pH	$\alpha$ -HCH	$\beta$ -HCH	$\gamma$ -HCH	$\delta$ -HCH	DDT	DDE	DDD	Chlorpyrifos	Malathion
1	Coca-Cola	UYF09305	2.53	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Pepsi	17320A041331	2.51	ND	ND	ND	ND	ND	ND	ND	ND	ND

#### Note:

1. Both the bottles were of plastic of 591ml capacity

2: ND - Not detected