ANALYSIS OF PESTICIDE RESIDUES IN SOFT DRINKS

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1. ABOUT THE CSE LABORATORY

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. PML is an ISO 9001:2000 certified laboratory, accredited by SWISO, Switzerland, doing pollution monitoring and conducting scientific studies on environmental samples. The Lab has qualified and experienced staff who exercise Analytical Quality Control (AQC) and meticulously follow what is called **Good Laboratory Practices**. It is equipped with state-of-art equipments for monitoring and analysis of air, water and food contamination, including Gas Chromatograph with Mass Detector (GC-MS), Gas Chromatograph (GC) with ECD, NPD, FID and other detectors, High Performance Liquid Chromatograph (HPLC), Atomic Absorption Spectrometer, UV-VIS Spectrophotometer, Mercury Analyzer, Respirable Dust Sampler etc. Its main aim is to undertake scientific studies to generate public awareness about food, water and air contamination. 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

After the publication of the study on pesticide residues in soft drinks in August 2003 by PML, a Joint Parliamentary Committee (JPC) was constituted by the Indian Parliament on "Pesticide Residues in and Safety Standards for Soft Drinks, Fruit Juices and Other Beverages" to check the veracity of the PML's study and to set the safety standards for soft drinks. In February 2004, JPC released its report in which it found PML's study to be correct and recommended the government to set pesticide standards for the soft drinks.

For the past three years, in 20-odd meetings, the Drinks and Carbonated Beverages Sectional Committee, FAD 14, of the Bureau of Indian Standards (BIS), has been deliberating on the issue of pesticide residue standards for soft drinks. This sectional committee includes representatives of all interested parties - industry (including Coca Cola and PepsiCo) and industry associations, food and nutrition scientists from the government scientific institutions, Union ministry of health, Union ministry of food processing and Union ministry of consumer affairs and consumer and environmental groups. In October 2005, the committee finalised the standards for pesticide residues in soft drinks. This final standard, which has not been notified yet, has set a limit of 0.1 ppb for individual pesticides and 0.5 ppb for total pesticides in soft drinks.

In December 2005, PML decided to check whether the samples of soft drinks available in the market are within the limits prescribed by BIS or not. A total of 57 samples, of 11 brands of Pepsi Co and Coca Cola available in the market, were collected from 12 different states of the country. PML tested the samples using internationally recognised United States Environment Protection Agency (USEPA) methodology for organochlorine pesticide and organophosphorus pesticide. Extraction and analysis was done as per the given methodology, using Gas Chromatograph with Electron Capture Detector using a capillary column for analysing organochlorine pesticides and Gas Chromatograph with Nitrogen Phosphorus Detector using a capillary column for analysing organophosphorus pesticides. Final confirmation was carried out by GC-MS.

3. SOFT DRINK INDUSTRY AND REGULATIONS

3.1 Definition of soft drink

Soft drinks are non-alcoholic water-based flavoured drinks that are optionally sweetened, acidulated and carbonated. Some carbonated soft drinks also contain caffeine; mainly the brown-coloured cola drinks.

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 the publication Global Soft drinks 2002, published by the Zenith International. Total volume reached 412,000 million litres in 2001, giving a global per capita consumption of around 67.5 litres per year.

GLOBAL SOFT DRINK MARKET Others Asia/Australia 14% 19% N America Latin America 27% 19% W Europe 21% **GLOBAL BEVERAGE SECTOR** Bottled water Carbonates 31% Soft drinks 45% Still drinks 10% Dilutables Juices/ 6% nectars 8%

GLOBAL SOFT DRINKS CONSUMPTION, 2001

Source: Zenith International, 2002 <u>www.globaldrinks.com</u>; viewed on BevNET.com (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 (Source: www.beveragemarketing.com/news2p.htm). Carbonated soft 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 the two multinational companies; Coca Cola and PepsiCo. Coca Cola leads the carbonated soft 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 take 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 flavoured drinks and sodas are popular in southern states. Western markets have preference towards mango-flavoured drinks.

Non-alcoholic beverage market can be divided into fruit drinks and soft drinks. Soft drinks available in glass bottles, aluminium 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 in Indian include brands like Pepsi Cola, Diet Pepsi, Coca- Cola, Diet Coke, Thumps Up etc. Cola drinks account for nearly 61-62% of the total soft drinks market in India. Non-Cola products account for 36% the total soft drink market (Source: India Infoline Sector Report, 2002).

Major Players in India

The two global majors PepsiCo and Coca-Cola dominate the soft drink market in India. Coca-Cola, which had winded 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. Some estimates put the market share of PepsiCo to be higher and some put the market share of Coca Cola to be higher. However, the soft drinks segment, dominated by these two companies, accounted for Rs 6,247 crore in sales in 2002.

3.3. Regulations

In India the soft drink 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.

In July 2004, the Union ministry of Health and Family Welfare issued a notification which stated that the water used in the manufacturing of soft drinks should meet the bottled water standard. But this notification remained silent on the final product standard for soft drinks.

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 Food Products Order (FPO), 1955. It regulates the general characteristics of a beverage. On the quality of basic raw materiel-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 for carbonated beverages in IS:2346:1992. The Joint Parliamentary Committee (JPC), set up in the wake of the PML's study on pesticide residues in soft drinks, asked BIS to revise the standards and come out with the standards on pesticide residues in soft drinks.

For the past three years, the Drinks and Carbonated Beverages Sectional Committee, FAD 14, of BIS has been deliberating on the issue of pesticide residue standards for soft drinks. This sectional committee includes representatives of all interested parties - industry (including Coca Cola and PepsiCo) and industry associations, food and nutrition scientists from the government scientific institutions, Union ministry of health, Union ministry of food processing and Union ministry of consumer affairs and consumer and environmental groups. In October 2005, the committee finalised the standards for pesticide residues in soft drinks. This final standard, which has not been notified yet, has set a limit of 0.1 ppb for individual pesticides and 0.5 ppb for total pesticides in soft drinks. But this BIS standard is voluntary and not mandatory in nature. Only those companies, that want to get the ISI certification will have to meet this standard.

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.

4. STANDARD FOLLOWED

The Drinks and Carbonated Beverages Sectional Committee, FAD 14, of the Bureau of Indian Standards (BIS) has set a limit of 0.1 ppb for individual pesticides and 0.5 ppb for total pesticides. This standard, which is finalised but still not notified, has been used by the PML in the present work. PML, therefore, used the finalised BIS limits set for single and multiple residues to compare the results of pesticide residue analysis in soft drinks.

5. LITERATURE REVIEW

There is a growing concern in the medical and scientific communities about the harmful effects associated with the consumption of carbonated soft drinks, especially by children, teenagers and vulnerable populations like pregnant women.

A common problem that is associated with the consumption of a large amount 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 stomach-ache as the stomach which maintains a very delicate acid-alkaline balance can be set out of balance by the consumption of large amount of soft drinks, which can create a constant acid state leading to indigestion and gassiness.

There are numerous medical and scientific studies that clearly document the harmful effects of some major ingredients of soft drinks, namely, carbon dioxide, artificial sweeteners like aspartame, saccharin, acesulfame-K etc., flavouring agents like caffeine, acids like phosphoric acid, some preservatives and excessive sugar.

Caffeine

A methylated xanthine, caffeine is a mildly addictive stimulant drug, used in soft drinks, as a "flavoring agent". 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 correlate exposure to caffeine during pregnancy to the occurrence of congenital malformations, foetal growth retardation, miscarriages (spontaneous abortions), behavioural effects and maternal fertility problems (Christian WS *et al,* 2001).

A new medical term "Caffeinism" has been coined to describe the health impact due to high intake of caffeine and it is a combination of agitation, disorientation, nervousness, twitching, recurrent headaches and gastrointestinal disturbances (Source: Eileen O'Connor, "A sip into dangerous territory," *Monitor on Psychology*, 32:5, June 2001).

In contrast to the claim made by the soft drink companies, scientific studies have found that caffeine has no role as a flavouring agent in the soft drinks. In a study conducted by the renowned Johns Hopkins Medicine (Johns Hopkins Hospital) in 2000 and funded by the National Institute on Drug Abuse, USA, it was found that, despite claims of the soft drink manufacturers, caffeine could not be detected as a flavour in soft drinks - and its use in soft drinks is more to create addiction to the soft drinks than flavour (<u>http://www.hopkinsmedicine.org/press/2000/august/000814.htm</u>).

The ill effect of caffeine can be gauged by the fact that the US FDA (US Food and Drug Administration) issued an advisory in 1981 warning that "Pregnant women should avoid caffeine-containing foods and drugs, if possible, or consume them only sparingly." The US FDA still maintains that advisory as its official policy.

The Australia and New Zealand Food Authority (ANZFA) in 2001 revised its standards for caffeine in soft drinks and carbonated beverages, keeping in mind the following "inherently cautious principles" to be used as a basis for regulatory decisions regarding caffeine.

These principles are:

- Where there is uncertainty regarding the potential for adverse health effects, particularly in children, a cautious
 approach should be taken regarding the broadening of any permission to add caffeine to food;
- When caffeine is added to a food other than one in which it occurs naturally, its presence, regardless of the source, should be identified on the label and, if appropriate, the approximate quantity in the product stated;
- Where caffeine is added to a food for its stimulant effect (i.e., at levels greater than necessary for flavoring), mandatory advisory statements that are additional to those applying to general foods would normally be required.

Artificial low-calorie sweeteners

Low-calorie sweeteners are non-sugar substances that are added to food and drink products instead of sugar. They have sweetness many times greater than conventional sugar. Artificial sweeteners like saccharin, aspartame and acesulfame-K have been linked with numerous diseases like cancer increasingly.

Saccharin has been linked in human studies to urinary-bladder cancer and in animal studies to cancers of the bladder and other organs (*Lancet* 1980;i:837-840. *Env. Health Perspectives* 1998; 25:173-200). Several cancer experts have questioned the safety of acesulfame-K, which was approved in 1998 for use in soft drinks in the USA. Acesulfame-K use in the soft drinks is also allowed under the Indian PFA, 1955.

Aspartame is a potent neurotoxin and endocrine disrupter. Carefully controlled clinical studies show that aspartame is not an allergen. However, certain people with the genetic disease phenylketonuria (PKU), those with advanced liver disease, and pregnant women with hyperphenylalanine (high levels of phenylalanine in blood) have a problem with aspartame because they do not effectively metabolise the amino acid phenylalanine, one of aspartame's components. High levels of this amino acid in body fluids can cause brain damage. Therefore, US FDA has ruled that all products containing aspartame must include a warning to phenylketonurics that the sweetener contains phenylalanine. This provision has also been included in the Indian PFA and needs to be strictly enforced.

The overall health impact of artificial sweeteners can be gauged by the fact that they are regulated by means of Acceptable Daily Intake (ADI), similar to toxic substances like pesticides. The ADI of major artificial sweeteners used in soft drinks is given below. It is to be noted that instead of one value for ADI, the ADI has been notified in a range from 0 (nil) to some value per kg body weight. The presence of 0 in ADI reflects the uncertainty in the ill effects of the sweeteners in susceptible individuals.

Low-calorie sweetener	Acceptable Daily Intake Level
 Acesulfame-K 	 0-15 mg/kg body weight per day
 Aspartame 	 0-40 mg/kg body weight per day
Cyclamate	 0-7 mg/kg body weight per day
Saccharin	 0-5 mg/kg of body weight per day

Source: FAO Nutrition Meetings Report Series, WHO/Food Additives; http://www.inchem.org/pages/jecfa.html

Sugar

It is well documented that diets high in refined sugar promotes obesity, which increases the risks of diabetes, high blood pressure, stroke, and heart disease. Sugary soft drinks also promote tooth decay. The high sugar content is a major reason why health professionals are concerned about frequent consumption of soft drinks.

The US Department of Agriculture recommends that a person who consumes a 2000 calorie diet should not consume more than about 40 grams of refined sugars per day. Children's by consuming one bottle of soft drink just about hit their daily recommended sugar limit from soft drinks alone. With normal food, candy, cookies, cake, ice cream, and other sugary foods, most exceed those recommendations by a large margin (Source: Michael F. Jacobson. "Liquid Candy: How Soft Drinks are Harming Americans' Health." Center for Science in the Public Interest, October 21, 1998, 4).

The change in the characteristics of soft drinks from occasional drink to a daily drink in urban India, especially among children and teenagers, warrants a serious look at the ill effects of consuming large quantities highly sugary soft drinks.

Acids and Carbon Dioxide

Dentists around the world are reporting complete loss of the enamel on the front teeth in teenaged boys and girls, who habitually drink soft drinks. The culprit is phosphoric acid in soft drinks, which causes tooth rot, as well as digestive problems and bone loss. Phosphoric acid has also been associated with calcium loss and kidney stones in numerous medical studies. 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 beverages because the amount of sugars that are consumed is important in forming caries caused by the bacteria *mutans streptococci*, 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.

A common problem that is associated with the consumption of a large quantity of soft drinks is the increased acid levels throughout the body causing gastronomic distress due to the inflammation of the stomach and erosion of the stomach lining leading to painful stomach ache 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. In this regard the use of acids like phosphoric acid and carbon dioxide has been studied in detail.

Carbon dioxide emitted from soft drinks is a waste product that humans excrete and can be harmful when ingested at high levels. 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 (<u>http://www.kauhawaii.com/softdrinks.html</u>).

Some of the important reports on the effects of soft drinks and additives on health and nutrition are listed below:

- Shanthy Bowman. Diets of individuals based on energy intakes from added sugars. Family Economics and Nutrition Review. 1999;12:31-8.
- Lisa Harnack, Jamie Stang, Mary Story. "Soft drink consumption among US children and adolescents: Nutrition consequences." Journal of the American Dietetic Association, April 1999. 99:4, 436-441.
- Amid Ismail, Brian Burt, Stephen Eklund, "The cariogenicity of soft drinks in the United States." Journal of the American Dental Association, August 1984,109:241-5.
- Amid Ismail, Jason Tanzer, Jennifer Dingle, "Current trends of sugar consumption in developing societies." Community Dentistry and Oral Epidemiology, 1997, 25:438-43.
- Marion Nestle, Michael Jacobson. "Halting the obesity epidemic: a public health policy approach." Public Health Reports. January/February 2000; 115:12-25. http://www.cspinet.org/reports/obesity.pdf
- David S. Ludwig, Karen E. Peterson, Steven L. Gortmaker, "Relationship Between Consumption of Sugarsweetened Drinks and Childhood Obesity: a Prospective, Observational Analysis." The Lancet. February 17, 2001, 505-8. http://www.commercialalert.org/candp/lancet.pdf
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- Michael F. Jacobson, Kelly D. Brownell. "Small Taxes on Soft Drinks and Snack Foods to Promote Health" American Journal of Public Health, 2000;90:854-7. http://www.cspinet.org/reports/tax/
- Grace Wyshak. "Carbonated Beverages, Dietary Calcium, the Dietary Calcium/Phospohorus Ratio, and Bone Fractures in Girls and Boys." Journal of Adolescent Health, 1994; 15:210-5.

6. MATERIALS AND METHODS

6.1. Sampling methodology

Soft drink bottles of different brands were purchased from various states of India during the month of December 2005 to April 2006. Extraction and pesticide residue analysis were carried out immediately after the samples were received at the lab. Fifty-seven samples of 11 soft drink brands were analysed for 15 organochlorines and 13 organophosphorus pesticides. The 15 organochlorines, which were tested, cover wide spectrum of chlorinated pesticides. The 13 organophosphorus pesticides tested are the most commonly used pesticides in India. Details of the samples purchased and tested by PML are given in Annexure I.

6.2. Equipments

Gas Chromatographs used for pesticide residue analysis were Thermoquest-Trace GC with the ⁶³Ni selective Electron Capture Detector with advanced software (Chromcard-32 bit Version 1.06 October 98) and Nucon GC-5765 series equipped with Nitrogen Phosphorus Detector. GC column employed were capillary column, DB-1701, J&W make and DB-210, J&W make respectively for organochlorine and organophosphorous pesticides. Rotary vacuum 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. Appropriate amounts of active ingredients were weighed accurately and individual stock solutions were prepared by dissolving it in hexane in a 40 ml brown bottle with a Teflon-lined screw cap. Appropriate aliquots of the obtained solutions were subsequently mixed into a 50 ml volumetric flask, which was filled to volume with hexane. Anhydrous sodium sulfate and sodium chloride was purchased from s. d. Fine Chem Ltd.

6.5.Sample Extraction and Clean up

The samples were analysed by using EPA Method 8081A for organochlorines 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 adjusted to neutral 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 sample was partitioned with 100 ml of methylene chloride (thrice) by shaking the separatory funnel vigorously for 2-3 minutes, 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.

Clean up

Cleanup was done by EPA Method 3620B - Florisil clean up by column chromatography. Florisil was activated at 130°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 20 mm ID chromatographic column. 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), then with 30%, 50% and 70% methylene chloride in hexane (100 ml each), and finally with methylene chloride (100 ml twice). Eluent was collected and concentrated to dryness. Final samples were prepared in hexane (HPLC grade) and analysed by GC-ECD for organochlorines and GC equipped with NPD for organophosphorus pesticides.

6.6. Sample Analysis

For Organochlorine pesticides

Organochlorines were analysed by Gas Chromatograph (Thermoquest-Trace GC) with the ⁶³ Ni selective electroncapture 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-1701 [(14%-Cyanopropyl-phenyl)-methylpolysiloxane (length 30m, ID 0.25 mm and film 0.25 μ m)]. The carrier gas and the makeup gas was nitrogen with a 0.5 ml/min and 30 ml/min-flow rate respectively employing the split less mode. 2.0 μ l of the final extract was injected at a temperature of 250°C. The oven temperature was kept at 150°C with a hold time of 1 minute, then from 150°C to 200°C at a rate of 10°C/minute with a hold time of 1 minute and then from 200°C to 250°C at a rate of 1°C/minute with a hold time of 1 minute of 4 minutes. The total run time was 65 minutes. The detector was maintained at 300^oC. Peak identification was performed by the GC software (Chromcard-32 bit Version 1.06 October 98) calibration table set up with a relative retention time window of 0.65%.

For Organophosphorus pesticides

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-210 [50% trifluoro propyl polysiloxane, 50% methyl polysiloxane (length 30m, ID 0.25 mm and film 0.25µm)]. The carrier gas and the makeup gas was nitrogen with a 2 ml/min and 30 ml/min-flow rate respectively, hydrogen at 8ml/min and air at 80ml/min were used employing the split less mode. 2.0µl of the final extract was injected at a temperature of 270°C. The oven temperature was kept at 120°C with a hold time of 1 minute, then from 120°C to 205°C at a rate of 25°C/minute with a hold time of 1 minute. The total run length was 51.4 minutes. The detector was maintained at 300°C.

The samples were calibrated (retention time, area count) against standard mixture of known concentration of 13 organophosphorus pesticides. Each peak was characterised by comparing relative retention time with those of standards. Analysis also included blank sample - a laboratory reagent blank of an aliquot of reagent water that was treated as a sample including exposure to all glassware equipment, solvents and reagents that were used, to determine if method analytes or other interferences are present in the laboratory experiment, the reagent or the apparatus.

6.7. Calculations

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

6.8. Recovery

A laboratory fortified sample matrix to which known quantities of the method analytes were added in the laboratory and analysed exactly like the sample to determine whether a sample matrix contributed bias to analytical results and to determine that methodology is in control was done. Recovery experiment was performed and recovery was about 75%-103% for organochlorines, about 59%-105% for organophosphorus pesticides. The reproducibility of results for all the pesticides was 95 percent and above for all the samples. However, the mean average reading of an individual sample analyzed in duplicate has been reported in the results.

S.No.	Organochlorine Pesticides	RT (min)	Recovery (%)	Organophosphorous Pesticide	RT (min)	Recovery (%)
1.	α-HCH	12.64	93.11	Dichlorvos	3.48	92.18
2.	ү-НСН	14.83	103.25	Acephate	5.17	85.17
3.	Heptachlor	15.98	97.81	Phorate	5.33	105.17
4.	Aldrin	17.73	82.98	Diazinon	6.18	98.79
5.	β-НСН	19.82	95.67	Chlorpyrifos	7.20	85.01
6.	δ-HCH	21.70	78.07	Fenthion	7.49	91.16
7.	α -Endosulfan	25.62	87.43	Dimethoate	8.00	90.36
8.	Cis-Chlordane	26.10	81.63	Monocrotophos	8.44	59.12

Table: 1 Percent recovery and retention time for organochlorine and organophosphorus pesticides

9.	Trans-Chlordane	26.85	74.94	Malathion	9.02	82.15
10.	DDE	27.68	94.39	Phenthoate	9.11	86.31
11.	Dieldrin	29.25	94.29	Fenitrothion	9.59	93.76
12.	DDD	36.63	99.62	Profenophos	10.53	92.93
13.	β-Endosulfan	37.13	102.88	Ethion	12.12	77.56
14.	DDT	38.64	82.66			
15.	Endosulfan Sulphate	46.65	79.80			

6.9. Confirmation and Quantification

Spiking

Identifications were confirmed by spiking the sample with known standard only to confirm the presence of residues.

GC-MS

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 split less injection of 2 μ l. The capillary column used was DB–1701 (30m x 0.25 mm x 0.25 μ m). The oven temperature was kept at 150°C with a hold time of 1 minute, then from 150°C to 200°C at a rate of 10°C/minute with a hold time of 1 minute and then from 200°C to 250°C at a rate of 1°C/minute with a hold time of 1 minute with a hold time of 4 minutes. The carrier gas was helium with a 0.5ml/min flow rate employing the split less mode. 2.0 μ l of the final extract (2ml) was injected at a temperature of 275°C keeping the ion source at 230°C; Multiplier: 1100 Volts; AGC: 50, 3 microscan; Default: Tune parameters: Autotune Tune File; Mass Range: 50- 650 m/z; SIM: Exact Mass +/- 0.5 amu.

7. RESULTS AND DISCUSSION

A total of 57 soft drinks samples of 11 brands – 7 brands of PepsiCo and 4 brands of Coca Cola – were tested for 15 organochlorine pesticides and 13 organophosphorus pesticides.

7.1. Organochlorine Pesticides

The average concentration of Total HCH ($\alpha+\beta+\gamma+\delta$) in all samples was 6.56 ppb. α -HCH was found in 21% samples; β -HCH in 14% samples; γ -HCH (Lindane) in 100% samples and δ -HCH in 100% samples. The range of concentration of Lindane in the 57 samples varied from 1.48 ppb - 14.06 ppb. Minimum concentration (1.48 ppb) was detected in Pepsi Caffechino (15 times the BIS limit) manufactured in Mathura, Uttar Pradesh and maximum concentration (14.06 ppb) was detected in Coca Cola, manufactured in Kolkata, West Bengal, which is 140 times the BIS limit for individual pesticides in soft drinks. Average concentration of Lindane detected in all the samples was 5.37 ppb, which is 52.7 times higher than the BIS limit for individual pesticides in soft drinks. (See ANNEXURE:II for details)

Hexachlorcyclohexane, previously called BHC (benzene hexachloride), is a mixture of eight isomers of which five are found in the crude product (α , β , γ , δ , ϵ). γ isomer of HCH was detected in 100% of soft drink samples, which might be because γ -HCH is more resistant to biological and chemical degradation under aerobic conditions (El beit *et al*, 1981) and is most commonly used. γ -HCH (Lindane) appears in the list of pesticides for restricted use in India (the use of Lindane formulations for 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. It is very effective against a wide variety of insects, including domestic insects and mosquitoes.

Heptachlor (banned with effect from September 20, 1996 in India) was detected in 72% of the samples at an average concentration of 0.41 ppb, which is 4 times the BIS limit for individual pesticides in soft drinks. The range of the concentration of Heptachlor in all samples varied from nil to 3.97 ppb.

DDT, Aldrin (banned with effect from September 20, 1996), Dieldrin (banned with effect from May, 1990), Endosulfan and Chlordane were not detected in any of the samples.

The range of concentration of total organochlorines in the 57 samples varied from 1.96 ppb - 18.94 ppb. Minimum concentration of 1.96 ppb was detected in a Coca Cola sample, manufactured in Haathras, Uttar Pradesh, and maximum concentration of 18.94 ppb was also detected in another Coca Cola sample, manufactured in Kolkata, West Bengal. Average concentration of total organochlorines was 6.97 ppb in all the 57 samples.

7.2. Organophosphorus Pesticides

Chlorpyrifos was detected in 100% of the samples analysed. The range of concentration in the 57 samples varied from 0.17 ppb to 20.43 ppb. Minimum concentration of 0.17 ppb was detected in a Limca sample, manufactured in Gandhinagar, Gujarat, and maximum concentration of 20.43 ppb was detected in a Coca cola sample, manufactured in Thane, Maharashtra, which is 200 times the BIS limit of 0.1 ppb for individual pesticides. Average concentration of chlorpyrifos in all 57 samples was 4.71 ppb, which is 47 times the BIS limit for the individual pesticide in soft drinks.

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

Malathion was present in 39% of the 57 samples analysed. The range of concentration in the 57 samples varied from nil to 3.11 ppb. Maximum concentration of 3.11 ppb, found in a Coca Cola sample manufactured in Hapur, Uttar Pradesh, is 31 times the BIS limit for the individual pesticide in soft drinks. The average concentration of malathion in all 57 samples was 0.17 ppb, which is 1.7 times the BIS limit for the individual pesticide in soft drinks.

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 anti-cholinesterase 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 organochorine pesticides; however they are relatively soluble in water and are highly toxic. They break down into non-toxic metabolites.

The range of concentration of total organophosphorus pesticides in the 57 samples varied from 0.17 ppb - 21.02 ppb. Minimum concentration of 0.17 ppb was detected in a Limca sample, manufactured in Gandhinagar, Gujarat and maximum concentration of 21.02 ppb was detected in a Coca Cola sample, manufactured in Thane, Maharashtra. Average concentration of organophosphorous pesticides was 4.88 ppb in all the 57 samples analysed.

7.3. Total pesticide residues

The range of concentration of total pesticides (organochlorines and organophosphorus) was 2.65 ppb to 31.55 ppb in all 57 samples. The Minimum concentration of 2.65 ppb, detected in a Thums Up sample manufactured in Thane, Maharashtra, is 5.3 times the BIS standard and the maximum concentration of 31.55 ppb, detected in a Coca Cola sample manufactured in Thane, Maharashtra, is 63.1 times the BIS limit for total pesticides in soft drinks (0.5 ppb). Average concentration of total pesticides detected in all 57 samples was 11.85, which is 24 times the BIS limit for total pesticides in soft drinks. (See Annexure: II for details).

7.4. Average Pesticide Residues - Brandwise

Total pesticide residues in all brands of PepsiCo products were 12.68 ppb, which is 25.4 times the BIS limit for total pesticides in soft drinks. In all brands of Coca-cola, the total pesticide residues were 11.05 ppb, which is 22.1 times the BIS limit for total pesticides in soft drinks. The highest concentration of pesticide residues was detected in Pepsi Cola, the flagship brand of PepsiCo. The total pesticide in Pepsi Cola was 15.2 ppb, which is 30.4 times the BIS standard for total pesticides. The lowest concentration was detected in Pepsi Caffechino, which is also a product of PepsiCo. The total pesticide in Pepsi Caffechino was 3.97 ppb, which is 7.9 times the BIS standard for total pesticides (see graph 1).



8. HEALTH IMPACTS OF PESTICIDES

The most common pesticides detected in the soft drink samples were – Lindane, δ -HCH, Chlorpyrifos, Heptachlor and Malathion. Health impacts of some of these pesticides are as follows:

8.1. Lindane

All isomers of HCH are stored in fats; the gamma isomer of HCH (Lindane), which was also found in soft dink samples, 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.

Lindane is a *potent carcinogen*. Rats exposed to gamma HCH showed evidence of liver cancer. (ATSDR, 1989). Chronic exposure to Lindane has been liked 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 dozes 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).

8.2. Chlorpyrifos

Chlorpyrifos, one of the most widely used organophosphorus pesticide and has been reported to be a developmental neurotoxicant specifically targeting the immature brain. (Barone *et al* 2000; Pope 1999) Foetal 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 in 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 *neuroterratogen*. 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 terratogenic effects* at maternal doze of 25 mg/kg per day, a doze that also produced maternal toxicity. Fetal weight and viability were decreased and fetal death and early resorption increased at this doze. (Farag AT *et al*, 2003). Studies carried out to evaluate potential toxicological effects of chlorpyrifos in rats showed that repeated exposure to sub threshold dozes of chlorpyrifos may lead to growth retardation, behavioral 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).

8.3. Malathion

Malathion, a known *cholinesterase inhibitor*, leads to 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 increse 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 fibroplasts after a single doze of 40 μ g/l or a double doze of 20 μ g/l. (Nicholas AH *et al*, 1979).

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 adenmatous patterns and ductal carcinomas with cribiform 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 malaoxon. (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 – malaoxon 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 ug/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).

8.4. Heptachlor

Heptachlor is a white crystalline solid with a mild camphor odour. It is used as an insecticide. Gas chromatography with electron capture detection is the method most commonly used for heptachlor determination. (Source: International Programme on chemical safety, Environmental Health Criteria 38, UNEP, WHO and ILO)

Heptachlor has been used for more than 30 years as a stomach and contact insecticide, mainly in the control of termites and soil insects. In its country of origin, the USA, its use is now restricted to underground termite control. In several other countries, approved uses have been gradually withdrawn. Exposure of the general population is mainly through residues in food.

Heptachlor is a persistent pesticide and bioaccumulation and biomagnification occur and bioconcentration factors of 200 - 37000X have been reported from water into hydro-biota. Heptachlor has been shown to be toxic for aquatic life, but its toxicity is highly species variable. Heptachlor is readily absorbed following ingestion and skin contact and is transported throughout the body. Heptachlor epoxide, the most persistent metabolite, is rapidly formed and can be found in the body, mainly in adipose tissue. The toxicity of heptachlor epoxide is similar to that of heptachlor.

According to the classification of Hodge & Sterner (1956), the acute toxicity of heptachlor is moderate (acute oral LD_{50} for the rat 40 - 162 mg/kg). WHO (1984) classified the technical product as moderately hazardous. Toxic symptoms are related to hyperexcitability of the central nervous system and include tremors and convulsions. Death may follow respiratory failure. At non-lethal acute exposures, heptachlor is hepatotoxic. Proliferation of the smooth endoplasmatic reticulum and induction of the mixed-function oxidases in liver cells is one of the earliest indications of prolonged exposure to heptachlor. At high exposure levels, heptachlor can interfere with reproduction and the viability of offspring. Cataracts were observed in both parents and progeny in the rat. There were no indications of teratogenicity in rats, rabbits, chickens, and beagle dogs. There is evidence that it may have effects on cell to cell communication, which is a property of promoting agents. There is limited evidence that both heptachlor and heptachlor epoxide are carcinogenic for mice.

9. CONCLUSION

From analysis of 57 samples of 11 different brands of soft drink samples it can be concluded that:

- Out of the 15 organochlorine and 13 organophosphorus pesticides analysed in the soft drink samples, Lindane, δ-HCH, Chlorpyrifos, Heptachlor and Malathion were most commonly found in 57 soft drink samples tested.
- Lindane (γ -Hexachlorocyclohexane), a potent carcinogen was detected in 100% of the samples analysed. The average concentration detected in all the samples was 5.37 ppb, which is 53.7 times the BIS 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.
- δ-HCH was also detected in 100% of the samples analysed. The average concentration detected in all the samples was 0.76 ppb, which is 7.6 times the BIS limit for individual pesticides.
- Heptachlor, a organochlorine pesticide banned for use in India, was detected in 72% of the samples at an average concentration of 0.41 ppb, which is 4.1 times the BIS limit for individual pesticides in soft drinks.
- Chlorpyrifos, a suspected neuroteratogen was detected in 100% of the 57 samples analysed with an average concentration of 4.71 ppb, which is 47 times the BIS limit.
- Malathion was present in 38.6% of the samples analysed with an average concentration of 0.17 ppb, which is 1.7 times the BIS limit.
- The average concentration of total organochlorines was 6.97 ppb and that of organophosphorus pesticides was 4.88 ppb.
- Total pesticide residues in the 57 soft drink samples manufactured in India was 11.85 ppb, which is 24 times the BIS limit for total pesticides in soft drinks (0.5 ppb).
- The variations in the pesticide residues in different samples could be due to the difference in ingredients, location of the manufacturing plants, difference in batches etc.

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<u>Annexure: I</u>

Details of soft drink samples analysed for pesticide residues

S. No.	Brand Name	Place of Manufacture	Batch Number	Date of manufacture	Expiry date	Place of Collection
1	Coca-Cola-1	Enrich Agro Food Product Ltd., 276-277, Udyog Vihar, Phase II, Dundahera, Gurgaon - 122016	BN 616	3/9/2005	Best before two and a half months from manufacture.	Gurgaon
2	Coca-Cola-2	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil-Hapur, Distt- Ghaziabad, UP	BN 1922 A2	7/9/2005	Best before two and a half months from manufacture.	Ghaziabad
3	CocaCola-3	Hindustan Coca-Cola Beverages Pvt. Ltd., Plot No. 18, Bidadi Indl Area, Bidadi Hobli, Ramnagar TQ, Bangalore - 562109	BN. 144	15/8/2005	Best before two and a half months from manufacture.	Bangalore
4	Coca-Cola-4	Hindustan Coca-Cola Beverages Pvt. Ltd., 44-69, Moula Ali, Hyderabad - 500400	BN 883	6/8/2005	Best before two and a half months from manufacture.	Hyderabad
5	Coca-Cola-5	Hindustan Coca-Cola Beverages Pvt. Ltd., Kaladera, Tehsil Chomun, Jaipur - 303801	BN 1965	13/9/2005	Best before three months from manufacture	Jaipur
6	Coca-Cola-6	Brindvan Beverages Pvt. Ltd., Parsakhera Industrial Estate, Bareilly (UP)	BN 95	19/4/2005	Best before two and a half months from manufacture	Nainital
7	Coca-Cola-7	Diamond Beverages Pvt. Ltd., P-41,Taratala Road, Kolkata - 700088	BNO 526	23/9/2005	Best before two and a half months from manufacture	Kolkata
8	Coca-Cola-8	Hindustan Coca-Cola Beverages Pvt. Ltd., Survey No. 284 P, Kudus, Tal Wada, Thane - 421312	BNO 379	7/12/2005	Best before two and a half months from manufacture.	Mumbai
9	Coca-Cola-9	Lakshmi Balaji Bottling Pvt. Ltd., S.No 131 Kapugunneri Vill, Srikalhasti Mandal, Chittoor - 517640	BN 523B	29/12/2005	Best before two and a half months from manufacture.	Jaipur
10	CocaCola-10	Kandhari Beverages Pvt. Ltd., Vill-Nabipur, Distt.Fatehgarh Sahib, Punjab -140406	BN. 9	12/1/2006	Best before two and a half months from manufacture.	Ludhiana
11	Coca-Cola-11	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	MG BN 107 A2	17/2/2006	Best before two and a half months from manufacture.	Delhi
12	Coca-Cola-12	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	MG BN307 A2	16/3/2006	Best before two and a half months from manufacture.	Ghaziabad
13	Coca-Cola-13	Brindavan Agro Industries Ltd., Vill -Nagla Ummed, Ruheri, Hathras, UP	BN 94	10/3/2006	Best before two and a half months from manufacture	Rishikesh
14	Coca-Cola-14	Hindustan Coca-Cola Beverages Pvt. Ltd, Burnihat, Meghalaya - 793101	BNO 780	17/2/2006	Best before two and a half months from manufacture.	Aizwal

S. No.	Brand Name	Place of Manufacture	Batch Number	Date of manufacture	Expiry date	Place of Collection
15	Fanta-1	Hindustan Coca-Cola Beverages Pvt. Ltd, Burnihat, Meghalaya - 793101	BNO 768	8/2/2006	Best before two and a half months from manufacture	Aizwal
16	Fanta-2	Surbhi Milkfood & Beveragaes Ltd., Kalol-Sanand Road, Vill - Moti bhoyan, Tal Kalol Distt, Gandhinagar - 382721	BN 005	27/2/2006	Best before three months from manufacture	Ahmedabad
17	Fanta-3	Brindavan Agro Industries Ltd., Vill-Nagla Ummed, Post-Ruheri, Hathras, UP	BN 034	16/2/2006	Best before three months from manufacture	Rishikesh
18	Fanta-4	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	MG BN 137 A2	22/2/2006	Best before two and a half months from manufacture	Delhi
19	Limca-1	Hindustan Coca-Cola Beverages Pvt. Ltd., Survey No 284 P, Kudus, Tal Wada, Distt.Thane - 421312	BNO 396	24/12/2005	Best before two and a half months from manufacture.	Mumbai
20	Limca-2	Kandhari Beverages Pvt. Ltd., Vill-Nabipur, Distt. Fatehgarh Sahib, Punjab -140406	BN 110	21/2/2006	Best before two and a half months from manufacture	Ludhiana
21	Limca-3	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	BN 51A2	28/1/2006	Best before two and a half months from manufacture	Delhi
22	Limca-4	Surbhi Milkfood & Beveragaes Ltd., Kalol-Sanand Road, Vill- Moti bhoyan, Tal kalol, Gandhinagar - 382721	BN 005	19/2/2006	Best before three months from manufacture	Ahmedabad
23	Limca-5	Hindustan Coca-Cola Beverages Pvt. Ltd, Kaladera, Tehsil- Chomun, Jaipur - 303801 Rajasthan	BNO 15	9/2/2006	Best before two and a half months from manufacture	Faridkot
24	Limca-6	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	MG BN 174 A2	26/2/2006	Best before two and a half months from manufacture.	Delhi
25	Thums-Up 1	Amrit Bottlers Pvt. Ltd., Vill -Chandpur Harbans, PO-Dabha Semar, Faizabad - 224133 (UP)	BN 466	4/7/2005	Best before two and a half months from manufacture	Gorakhpur
26	Thums Up-2	Hindustan Coca-Cola Beverages Pvt. Ltd., Plot No 181, Bidadi Indl. Area, Bidadi Hoobli, Ramnagar TQ, Bangalore - 562109 Karnataka	PKB NO 35	25/1/2006	Best before three months from manufacture	Ahmedabad
27	Thums Up-3	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	BN 73 A2	4/2/2006	Best before two and a half months from manufacture	Delhi
28	Thums Up-4	p-4 Hindustan Coca-Cola Beverages Pvt. Ltd., Survey No 284 P, Kudus, Tal Wada, Distt.Thane - 421312 Maharashtra		14/2/2006	Best before two and a half months from manufacture	Jhabua
29	Thums Up-5	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Ghaziabad, UP - 201009	BNO 32	17/2/2006	Best before two and a half months from manufacture	Faridkot
30	Pepsi Cola -1	Varun Beverages Ltd, Dautana, Chatta Distt, Mathura - 282401	B 549	14/9/2005	Best before three months from manufacture.	Gurgaon

S. No.	Brand Name	Place of Manufacture	Batch Number	Date of manufacture	Expiry date	Place of Collection
31	Pepsi Cola-2	Pepsico India Holdings Pvt. Ltd., 34th KM Stone, N.H 4, Teppada Begur, Nelamangala, Bangalore - 562123	B.NO-621	16/9/2005	Best before three months from manufacture.	Bangalore
32	Pepsi Cola -3	SMV Beverages Pvt. Ltd., A-28 MIDC Industrial Area, Saoner, Nagpur - 441107	B 488	14/9/2005	Best before three months from manufacture.	Hyderabad
33	Pepsi Cola-4	Varun Beverages Ltd, Plot No. Special-159, Riico Industrial Area, Phase-III, Boranada, Jodhpur - 342001 (Rajasthan)	B 187	8/9/2005	Best before three months from manufacture.	Jaipur
34	Pepsi Cola-5	Pepsico India Holdings Pvt. Ltd., A-2, UPSIDC Industrial Area, Jainpur, Kanpur Dehat - 209311	W-41P334	19/9/2005	Best before three months from manufacture	Gorakhpur
35	Pepsi Cola-6	Pepsico India Holdings Pvt. Ltd., JL-47, Barhans Fartabad, Charaktala, 24 Paraganas (WB) 700084	P 171	15/9/2005	Best before three months from manufacture.	Kolkata
36	Pepsi Cola-7	Pepsico India Holdings Pvt. Ltd , Offsion Trombay Road, Chembur, Mumbai - 400088.	B 2912	29/12/2005	Best before three months from manufacture.	Mumbai
37	Pepsi Cola-8	Pepsico India Holdings Pvt. Ltd., 34th KM Stone, N.H 4, Teppada Begur, Nelamangala, Bangalore - 562123	B.NO 803	30/11/2005	Best before three months from manufacture.	Bangalore
38	Pepsi Cola-9	Pepsico India Holdings Pvt. Ltd., 27,GIDC, Jhagadia, Bharuch - 393110	B 212	23/12/2005	Best before three months from manufacture	Ahmedabad
39	Pepsi Cola-10	SMV Beverages Pvt. Ltd., A-28, MIDC Growth Centre, Saoner Distt, Nagpur - 441107 (Maharastra)	BNO-544	31/12/2005	Best before three months from manufacture.	Mumbai
40	Pepsi Cola-11	Pepsico India Holdings Pvt. Ltd., 34th KM Stone, N.H 4, Village Teppada Begur, Nelamangala, Bangalore - 562123	BNO 003	4/1/2006	Best before three months from manufacture.	Bangalore
41	Pepsi Cola-12	Dhillon Kool Drinks and Beverages Pvt. Ltd., G.T.Road, Phillaur, Distt. Jalandhar - 144410	BNO 103 L1	22/2/2006	Best before three months from manufacture	Ludhiana
42	Pepsi Cola-13	Varun Beveragaes Ltd., 107 KM Stone, Agra-Delhi N.H.2, Vill- Dautana, Chatta - 281401, Mathura	B-40	11/2/2006	Best before three months from manufacture.	Rishikesh
43	Pepsi Cola -14	Dhillon Kool Drinks and Beverages Pvt. Ltd., G.T.Road, Phillaur, Jalandhar -144410 Punjab	BNO 147 L1	4/3/2006	Best before three months from manufacture.	Faridkot
44	7-UP-1	Pepsico India Holdings Pvt. Ltd., Wise Park Indl. Devt. Area, Kanjikode East PO, Palakkad - 678621		11/1/2006	Best before three months from manufacture	Palakkad
45	7-UP-2	Pepsico India Holdings Pvt. Ltd., 34th KM Stone, N.H 4, Teppada Begur, Nelamangala, Bangalore - 562123	BNO 853	28/12/2005	Best before three months from manufacture	Bangalore
46	7-UP-3	Pepsico India Holdings Pvt. Ltd., 27,GIDC, Jhagadia, Bharuch - 393110	B-06	1/2/2006	Best before three months from manufacture	Ahmedabad
47	Mirinda Orange-1	SMV Beverages Pvt. Ltd., A-28, MIDC Growth Centre, Saoner Distt, Nagpur - 441107 (Maharastra)	BNO 543	29/12/2005	Best before three months from manufacture	Mumbai

S. No.	Brand Name	Place of Manufacture	Batch Number	Date of manufacture	Expiry date	Place of Collection
48	Mirinda Orange-2	Pepsico India Holdings Pvt. Ltd., 27,GIDC, Jhagadia, Bharuch - 393110 Gujarat	B 60	24/12/2005	Best before three months from manufacture	Ahmedabad
49	Mirinda Orange-3	Dhillon Kool Drinks and Beverages Pvt. Ltd., G.T.Road, Phillaur, Distt. Jalandhar -144410 (Punjab)	BNO 120 L1	25/2/2006	Best before three months from manufacture	Ludhiana
50	Mirinda Orange-4	Pepsico India Holdings Pvt. Ltd., 27,GIDC, Jhagadia, Bharuch - 393110	B 07	21/2/2006	Best before three months from manufacture	Ahmedabad
51	Mirinda Orange-5	Pepsico India Holdings Pvt. Ltd., Offsion, Trombay Road, Chembur, Mumbai - 400088 Maharashtra	B 2001	20/1/2006	Best before three months from manufacture.	Jhabua
52	Mirinda Orange-6	North-East Pure Drinks Pvt. Ltd., Rani Patgaon, Guwahati - 781017	L1 BN 10	17/2/2006	Best before three months from manufacture.	Guwahati
53	Mirinda Orange-7	393110Dhillon Kool Drinks and Beverages Pvt. Ltd., G.T.Road, Phillaur, Distt-Jalandhar -144410	BNO 150 L1	4/3/2006	Best before three months from manufacture	Faridkot
54	Duke Lemonade	Pepsico India Holdings Pvt. Ltd., Offsion, Trombay Road, Chembur, Mumbai - 400088 Maharashtra.	B 1602	16/2/2006	Best before three months from manufacture	Jhabua
55	Mirinda-Lemon	Pepsico India Holdings Pvt. Ltd., 27,GIDC, Jhagadia, Bharuch - 393110	147	19/3/2006	Best before three months from manufacture	Ahmedabad
56	Mountain dew	Varun Beveragaes Ltd., 107 KM Stone, Agra-Delhi N.H.2, Vill- Dautana, Chatta - 281401, Distt.Mathura UP	В 14	7/3/2006	Best before three months from manufacture	Rishikesh
57	Pepsi Café Chino	Varun Beveragaes Ltd., 107 KM Stone, Agra-Delhi N.H.2, Vill- Dautana, Chatta - 281401, Distt. Mathura UP	B 09	21/2/2006	Best before three months from manufacture.	Rishikesh

Annexure: II

Organochlorine and organophosphorous pesticide in soft drink samples

S. No.	Sample Details	Pestici	de Resi	dues in	parts pe	er billion (pp	ob)						
		Organo	ochlorin	e Pestic	ides				Organophos	phorous Pe	sticides	Total	No. of times the BIS
		α-HCH	β-НСН	ү-НСН	δ-НСН	Total HCH	Heptachlor	Total Organochlorines	Chlorpyrifos	Malathion	Total Organophosphates	Pesticides	standard for total pesticides in soft drinks (0.5 ppb)
1	Coca Cola 1	0.00	0.00	3.60	0.37	3.98	0.00	3.98	0.77	0.00	0.77	4.75	9.5
2	Coca Cola 2	0.00	0.00	9.27	0.55	9.82	0.00	9.82	1.62	3.11	4.73	14.55	29.1
3	Coca Cola 3	0.00	0.00	3.77	0.30	4.07	0.00	4.07	2.49	0.00	2.49	6.56	13.1
4	Coca Cola 4	0.00	0.00	5.78	0.45	6.23	0.00	6.23	2.15	0.00	2.15	8.38	16.8
5	Coca Cola 5	1.82	0.00	7.79	1.78	11.39	0.00	11.39	10.45	0.00	10.45	21.84	43.7
6	Coca Cola 6	1.18	0.00	5.23	1.44	7.84	0.00	7.84	13.27	0.00	13.27	21.11	42.2
7	Coca Cola 7	2.99	0.00	14.06	1.89	18.94	0.00	18.94	9.57	0.00	9.57	28.51	57.0
8	Coca Cola 8	0.00	0.00	6.96	3.16	10.12	0.41	10.53	20.43	0.60	21.02	31.55	63.1
9	Coca Cola 9	0.00	0.00	4.13	0.70	4.83	1.43	6.26	6.41	0.00	6.41	12.68	25.4
10	Coca-Cola 10	0.00	0.00	5.20	0.82	6.02	0.12	6.14	4.35	0.41	4.77	10.91	21.8
11	Coca Cola 11	0.00	0.00	2.23	0.49	2.71	0.26	2.97	2.00	0.00	2.00	4.97	9.9
12	Coca Cola 12	0.00	0.06	3.83	0.10	3.99	0.20	4.19	0.24	0.13	0.37	4.56	9.1
13	Coca Cola 13	0.00	0.00	1.59	0.11	1.70	0.26	1.96	7.39	0.00	7.39	9.35	18.7
14	Coca Cola 14	0.00	0.00	2.53	0.45	2.98	0.27	3.26	4.39	0.00	4.39	7.64	15.3
15	Fanta 1	0.00	0.00	5.44	0.08	5.51	0.29	5.80	0.35	0.25	0.60	6.40	12.8
16	Fanta 2	0.00	0.15	2.66	0.66	3.48	0.59	4.06	4.17	0.36	4.53	8.59	17.2
17	Fanta 3	0.00	0.00	9.25	0.42	9.67	0.43	10.09	2.56	0.36	2.92	13.02	26.0
18	Fanta 4	0.00	0.00	1.67	0.30	1.97	0.50	2.47	6.10	0.00	6.10	8.56	17.1
19	Limca 1	0.00	0.00	3.87	0.15	4.02	1.13	5.16	2.89	0.15	3.04	8.20	16.4
20	Limca 2	0.00	0.00	2.88	0.47	3.35	0.12	3.47	4.13	0.15	4.28	7.75	15.5
21	Limca 3	0.00	0.00	3.94	0.68	4.62	0.15	4.77	5.43	0.22	5.65	10.42	20.8
22	Limca 4	0.00	0.00	1.82	2.35	4.17	0.09	4.26	0.17	0.00	0.17	4.42	8.8
23	Limca 5	0.00	0.00	4.24	0.65	4.89	0.14	5.03	2.58	0.00	2.58	7.61	15.2
24	Limca 6	0.00	0.00	2.52	0.47	2.99	0.27	3.26	0.20	0.00	0.20	3.46	6.9
25	Thums Up 1	2.13	0.00	10.78	2.45	15.36	0.00	15.36	0.44	0.00	0.44	15.80	31.6
26	Thums Up 2	0.00	0.00	8.70	0.86	9.57	0.00	9.57	6.85	0.16	7.01	16.57	33.1

S. No	.Sample Details	Pestici	de Resid	dues in	parts pe	er billion (pp	b)						
		Organochlorine Pesticides							Organophos	Organophosphorous Pesticides			No. of times the BIS
		α-НСН	β-НСН	ү-НСН	δ-НСН	Total HCH	Heptachlor	Total Organochlorines	Chlorpyrifos	Malathion	Total Organophosphates	Pesticides	standard for total pesticides in soft drinks (0.5 ppb)
27	Thums Up 3	0.00	0.00	6.85	0.77	7.62	0.15	7.78	5.54	0.38	5.92	13.70	27.4
28	Thums Up 4	0.00	0.00	1.81	0.24	2.06	0.36	2.42	0.23	0.00	0.23	2.65	5.3
29	Thums Up 5	0.00	0.00	2.21	0.33	2.54	0.27	2.81	3.15	0.00	3.15	5.96	11.9
30	Pepsi Cola 1	0.24	0.00	8.44	0.49	9.18	0.00	9.18	0.57	0.00	0.57	9.75	19.5
31	Pepsi Cola 2	4.46	0.00	12.51	0.74	17.71	0.00	17.71	11.56	0.00	11.56	29.27	58.5
32	Pepsi Cola 3	2.87	0.00	11.62	0.59	15.07	0.00	15.07	0.79	0.00	0.79	15.87	31.7
33	Pepsi Cola 4	0.00	0.00	5.31	0.34	5.65	0.00	5.65	18.62	0.00	18.62	24.26	48.5
34	Pepsi Cola 5	3.68	0.00	13.03	2.00	18.71	0.00	18.71	7.41	0.00	7.41	26.12	52.2
35	Pepsi Cola 6	2.90	0.00	11.62	1.73	16.26	0.00	16.26	6.93	0.00	6.93	23.19	46.4
36	Pepsi Cola 7	0.00	0.00	3.07	0.41	3.48	1.54	5.02	6.57	0.19	6.76	11.77	23.5
37	Pepsi Cola 8	0.00	0.04	2.95	0.17	3.16	1.67	4.84	3.14	0.00	3.14	7.98	16.0
38	Pepsi Cola 9	0.00	0.22	3.98	0.86	5.06	0.38	5.45	6.02	0.00	6.02	11.47	22.9
39	Pepsi Cola 10	0.00	0.00	4.32	0.78	5.10	3.97	9.07	6.00	0.24	6.23	15.30	30.6
40	Pepsi Cola 11	0.41	0.00	2.97	1.01	4.39	0.77	5.16	6.45	0.00	6.45	11.61	23.2
41	Pepsi Cola 12	0.44	0.00	3.81	0.87	5.12	0.20	5.32	6.03	0.35	6.39	11.70	23.4
42	Pepsi Cola 13	0.00	0.00	4.94	0.26	5.20	0.18	5.38	2.27	0.00	2.27	7.64	15.3
43	Pepsi Cola 14	0.00	0.00	2.51	0.47	2.98	1.71	4.69	2.42	0.00	2.42	7.11	14.2
44	7-UP 1	0.00	0.04	4.67	0.33	5.04	1.33	6.37	3.41	0.00	3.41	9.78	19.6
45	7-UP 2	0.00	0.24	4.79	0.61	5.64	0.12	5.75	11.49	0.00	11.49	17.24	34.5
46	7-UP 3	0.00	0.18	4.68	0.65	5.51	0.08	5.59	4.69	0.09	4.79	10.37	20.7
47	Mirinda Orange 1	0.00	0.00	7.85	2.50	10.34	0.31	10.66	8.20	0.35	8.55	19.21	38.4
48	Mirinda Orange 2	0.63	0.00	4.02	1.09	5.75	0.00	5.75	6.12	0.00	6.12	11.86	23.7
49	Mirinda Orange 3	0.00	0.00	5.42	1.03	6.45	0.20	6.65	5.90	0.35	6.26	12.91	25.8
50	Mirinda Orange 4	0.00	0.00	6.83	0.12	6.95	0.30	7.26	0.34	0.53	0.86	8.12	16.2
51	Mirinda Orange 5	0.00	0.00	10.82	0.19	11.01	0.42	11.42	0.49	0.56	1.06	12.48	25.0
52	Mirinda Orange 6	0.00	0.00	3.26	0.15	3.41	0.36	3.77	0.53	0.31	0.84	4.61	9.2
53	Mirinda Orange 7	0.00	0.00	1.71	0.22	1.93	0.45	2.38	3.20	0.00	3.20	5.58	11.2
54	Duke Lemonade	0.00	0.00	5.56	1.20	6.75	0.97	7.72	3.16	0.00	3.16	10.88	21.8
55	Mirinda Lemon	0.00	0.05	6.40	0.27	6.72	0.40	7.12	0.52	0.45	0.96	8.09	16.2
56	Mountain Dew	0.00	0.00	2.71	0.55	3.26	0.34	3.60	3.37	0.00	3.37	6.97	13.9
57	Pepsi Caffechino	0.00	0.00	1.48	0.25	1.73	0.23	1.96	2.02	0.00	2.02	3.97	7.9

ANNEXURE-III GC-MS — Spectra



GC-MS Spectra — Sample

Hit	<u>SI</u>	RSI	Prob	Name	Library Name
1	590	590	96.74	MALATHION; [MALDISON], [MALATHON], [MERCAPT	
2	545	545	96.74	MALATHION; [MALDISON], [MALATHON], [MERCAPT	
3	362	464	0.96	CYTHIOATE: [O.O-DIMETHYL O-4-SULFAMOYLPHENY	PESTICIDE
4	354	427	0.72	TEPP:[ETHYL PYROPHOSPHATE]: (Nifos T). (Vapotone	PESTICIDE
5	348	458		ANILINE 3-CHLORO	PESTICIDE
6	334	390	0.35	MALAOXON	PESTICIDE
7	316	428	0.18	ANILINE 4-CHLORO	PESTICIDE
8	314	442	0.16	CHLORBENSIDE: (Chlorparacide), (Chlorsulphacide	PESTICIDE
9	302	425	0.16	CHLORBENSIDE: (Chlorparacide), (Chlorsulphacide	PESTICIDE
10	298	419	0.09	DRAZOXOLON	PESTICIDE
11	298	338	0.09	DINOBUTON; (Acrex), (Sytasol)	PESTICIDE
12	269	314	0.35	MALAOXON	PESTICIDE
13	250	266	0.02	DIFLUBENZURON; (Dimilin)	PESTICIDE
14	242	259	0.35	MALAOXON	PESTICIDE
15	239	260	0.01	HEPTENOPHOS: (Hostaquick), (Ragadan) YRIC ACIE	PESTICIDE



GC-MS Spectra — Standard



GC-MS Spectra — Sample



GC-MS Spectra - Sample



GC-MS Spectra - Standard



GC-MS Spectra - Sample



GC-MS Spectra — Standard



GC-MS Spectra - Sample



GC-MS Spectra — Standard