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Leaching of styrene and other aromatic compounds in drinking water from PS bottles

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Abstract

Bottled water may not be safer, or healthier, than tap water. The present studies have proved that styrene and some other aromatic compounds leach continuously from polystyrene (PS) bottles used locally for packaging. Water sapmles in contact with PS were extracted by a preconcentration technique called as "purge and trap" and analysed by gas chromatograph-mass spectrometer (GC/MS). Eleven aromatic compounds were identified in these studies. Maximum concentration of styrene in PS bottles was 29.5 μ g/L. Apart from styrene, ethyl benzene, toluene and benzene were also quantified but their concentrations were much less than WHO guide line values. All other compounds were in traces. Quality of plastic and storage time were the major factor in leaching of styrene. Concentration of styrene was increased to 69.53 μ g/L after one-year storage. In Styrofoam and PS cups studies, hot water was found to be contaminated with styrene and other aromatic compounds. It was observed that temperature played a major role in the leaching of styrene monomer from Styrofoam cups. Paper cups were found to be safe for hot drinks.

Key words: styrene; polystyrene (PS); ethyl benzene; leaching; styrofoam

Introduction

Bottled water is now so popular that there are more than 700 brands of water worldwide (Gordon, 2001). Almost all type of plastics is used for bottling purposes. Even polystyrene (PS) is also used for small packaging. An estimate is that 1.5 million tones of plastic is used yearly by the bottled water industry. Buying bottled water is not a long-term sustainable solution to have access to healthy water. The plasticizer start migration from day one, destruction processes, aging of plastics and the presence of unbound low-molecular-mass compounds considerably increase migration level. The extent to which migration occurs depending upon such factors as the contact area, type of plastic, temperature, and contact time. The U.S. Food and Drug Administration (FDA) call these plastics "Food Contact Substances" (FDA, 2004).

Rigid PS and PS-related plastics, which are used as food packaging materials, have longer history of use than poly vinyl chloride (PVC). Some of the physical characteristics of PS, for example, its low impact strength and chemical resistance, have led to the development of other food grade plastics in which styrene is co-polymerized with monomers like butadiene and acrylonitrile to give it more flexibility. Styrofoam is cross-linked PS, which is blown out using blowing agent during manufacture. The Styrofoam that keeps coffee hot and eggs from breaking is an economical product used in everything from packing peanuts to sanitary ware. It is used not only in the rigid molded state as food containers (dairy products) but is now manufactured in a form suitable for use as reusable cutlery and in the foamed state, as drink containers (for hot drinks).

The fact that styrene can adversely effect humans in a number of ways raises serious public health and safety questions regarding its build-up in human tissue and the root cause of this build-up. Long-term exposure to small quantities of styrene can cause neurotoxic (fatigue, nervousness, sleeplessness), hematological (low platelet and hemoglobin values), cytogenetic (chromosomal and lymphatic abnormalities), and carcinogenic effects (Dowty et al., 1976). In 1987, the International Agency for Research on Cancer (IARC), re-classified styrene from a Groups 3 (not classifiable as to its carcinogenicity) to a Group 2B substance (possibly carcinogenic to humans) (ATSDR, 1992). There are evidences that styrene is a carcinogen and neurotoxin (Dowty et al., 1976), and it has also been linked to reproductive problems. Styrene is also found in the milk of feeding females (Brown, 1991). Women exposed to low concentrations of styrene vapors in the workplace are known to have a variety of neurotoxic and menstrual problems. Certain styrene oligimers have proliferative activity on MFC-7 human breast tumor cells and binding affinity for human estrogen receptor α (Ohyama et al., 2001). Styrene is mainly biotransformed to styrene-7,8-oxide via the mixed function oxidase system. It has been reported

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(Van Duuren, 1969; Cohen *et al.*, 2002) that styrene-7,8oxide bind with DNA and induces tumors and probably causes carcinogenicity.

Styrene is a contaminant in all PS foam packages. It was first documented in 1972 (Figge, 1972) and then again in 1976 (Dowty *et al.*, 1976). Styrofoam leach styrene into the liquids they contain. Leaching of styrene increased by heating (Withey, 1976). Styrene has been found in food, packed in PS containers (FDA, 2003; Health Canada, 1993). Styrene has been reported in yogurt, cream, salads, soft cheese, margarines, hot or cold beverage, fresh and cooked meat, candied fruit and fast food packed in PS (Miller *et al.*, 1994). Styrene level has also been measured in human tissue. According to a study (ASTDR, 1992) styrene was detected in adipose tissues, and blood.

Considering the importance of toxic effect of styrene and its leachability in food materials and water from PS, the present studies were conducted. Water samples packed in PS bottles and styrofoam, PS and paper cups were collected from local market of Yanbu Industrial City (Saudi Arabia). Apart from the packing and date of expiry no other record of these bottled water was available. All these samples were assayed for styrene monomer contents.

1 Materials and methods

1.1 Sample collection and preservation

All the water samples in PS bottles were collected from local market and were analyzed on the same day. PS disposable cups, paper cups and styrofoam cups were also purchased from local market: PS bottles filled with water (250-ml); styrofoam cups (250-ml); PS disposable cups (80-ml); paper cups (250-ml). Before analysis pH of the each sample was also measured and it was: bottled water 7.2 to 7.5; potable water 7.9 to 8.1.

1.2 Analytical techniques

Modern chromatographic techniques including capillary gas chromatography and high-performance liquid chromatography (HPLC) together with highly selective detectors like flame ionization (FID), electron capture (ECD), nitrogen-phosphorous (NPD), mass spectrometer (MS), ultraviolet (UV), electrochemical and fluorescent, ensure separation, identification, and precise determination of the majority of toxic substances migrating from plastics to contact media at the levels required for safety evaluation. Usually sample preparation is performed by a number of different analytical techniques, including purge and trap (P&T) (Munch, 1995), liquid-liquid extraction (LLE), solid phase extraction (SPE), headspace (Withey, 1976) and direct aqueous injection in combination with a chromatographic system. In the present study samples were extracted and preconcentrated on VOCARB® 3000 trap by Tekmar Velocity XPT accelerated purge and trap sample concentrator and analyzed by Varian 3800 Gas chromatograph and Saturn 2200 mass spectrometer (GC/MS). USEPA method 524.2 (Munch, 1995) was followed as guideline for extraction.

1.3 Operating conditions for purge and trap and GC/MS

Optimized operating conditions for purge and trap are given below: transfer line temperature 150°C; desorption temperature 250°C; purge time 11 min; desorption time 2.0 min; purge temperature ambient; desorption flow 300 ml/min; purge flow 40 ml/min; bake temperature 300°C.

Detailed condition for GC temperature programming, column, and MS parameters are given below: injector temperature 200°C; carrier gas (helium) 99.9999%; carrier gas flow: 1.0 ml/min; transfer line temperature 250°C; emission current 20 μ A; ionization mode: EI; column: FactorFour Capillary column VF-5MS 30 m × 0.25 mm ID, df=0.25 Varian; column temperature 35°C (3 min) to 150°C (1.0 min) at 12.5 °C/min then to 280°C (2 min) at 30°C/min.

1.4 Reagents

Reagent water: reagent water was generated from Millipore Milli Q ultra pure water purification unit; stock solutions: stock standard solutions of 1000 mg/L in methanol from Supelco; sub stock solution: 200 mg/L in methanol; working standards: 2.5, 10, and 20 mg/L in methanol.

Velocity XPT purge and trap was connected with gas chromatograph, calibrated the P&T-GC system using high purity nitrogen as purging gas. Each calibration standard was extracted and preconcentrated by directly injecting (2.0 μ l of working standard mixture in the syringe containing 5 ml reagent water) in the sparger under the optimized condition and analyzed by GC/MS. For quantification purposes qualifier ion and retention time (RT) for each compound were used. For benzene, toluene, ethyl benzene and styrene qualifier ions were 78, 91, 91 and 104 and RT 3.549, 5.352, 7.030 and 7.556 min respectively. Reagent water (5.0 ml) spiked with 2 μ l working standards (2.5, 10.0 and 20.0 mg/L) was equivalent to 1.0, 4.0, and 8.0 μ g/L of each compound respectively.

Concentration of individual compound was determined by peak area using software MS Workstation Version 6.4. Response factors (RF) for each compound was calculated and three point calibration curves were prepared.

1.5 Experimental method

The details of different experiments are as follows: (1) bottled water samples were analyzed within 4 h after collection; (2) in styrofoam and paper cups studies, 200 ml boiling water was added in the cups and kept at ambient temperature for 60 min; (3) in PS disposable cups, 70 ml boiling water was added and kept at ambient temperature for 60 min.

After optimizing GC/MS and purge and trap conditions and preparing calibration curves, 5 ml water sample was carefully sucked into the syringe and injected into sparger. High purity nitrogen (99.999%) was purged at 40 ml/min for 11 min. Purged volatiles were preconcentrated on VOCARB[®] 3000 trap. Then dry air was purged to remove moisture. Trap temperature was increased to 250°C and adsorbed volatiles were desorbed and transferred to GC injector through a preheated transfer line with high purity (99.9999%) helium gas. Analysis was performed by GC/MS and concentration of individual component was calculated by calibration curve. Reference spectra of all the identified compounds produced by GC/MS system were recorded.

2 Results and discussion

2.1 Bottled water

The main work was to assess the extent to which styrene is leached in water from PS material. In this study water in PS bottles was found to contain styrene and some other aromatic compounds (Table 1). Results show that styrene was significantly high in all PS bottled water. Locally three brands of PS bottled water were available and all were found contaminated with styrene, toluene, ethyl benzene and benzene. Maximum concentration was of styrene (Table 1). Concentration of other identified aromatic compounds was much less than styrene.

Presence of styrene in water is supposed to be due to the leaching of free styrene monomer present in the PS plastic. As it has been reported that styrene monomer present in PS ranges from 300 to 1000 mg/kg (EU, 2002). The FDA requires that residual styrene monomer level in basic styrene polymer products intended for fatty food should not be more than 0.5% (5000 mg/kg) (FDA, 2004). The 2nd major compound found in water stored in PS was ethyl benzene. Its concentration ranged from 0.42 to 3.21 μ g/L. As it is known that styrene is manufactured from ethylene benzene and traces of ethyl benzene are always there. Impurities vary by plant and production method and can include ethyl benzene (<0.1%), isopropyl benzene, toluene, benzene, p-xylene, 2-phenyl propene etc. (EU, 2002). Presence of ethyl benzene, toluene and benzene indicates that these impurities were present in styrene used for the production of PS. These impurities also leached along with styrene in the water. Presence of styrene in food material and water packed in PS containers is also reported in literature (EU, 2002; Withey, 1976; FDA, 2003; Paul, 2003). Guideline value by WHO (2004) and maximum permissible limits by Royal Commission (RC) for Jubail and Yanbu, Saudi Arabia (RC, 2004) are given in Table 2. Maximum concentration of styrene in this study was 29.5 µg/L, higher than World Health Organization (WHO) guide line values and RC maximum permissible

Table 2 WHO/RC maximum permissible limits (µg/L)

| Compound name | WHO limits | RC limits | |
|---------------|------------|-----------|--|
| Benzene | 10 | 10 | |
| Ethyl benzene | 300 | 300 | |
| Toluene | 700 | 700 | |
| Styrene | 20 | 20 | |

limits (20 µg/L).

Correlation of results with packing date labeled on the bottle (Table 1) indicates that storage time have significant effect on leaching of monomers. Further studies were conducted to investigate the effect of storage time. Three bottles of the same brand with same packing date and batch number were purchased from the market. Packing date on all the three bottles was April 2005, the first analysis was done on 9 June 2005. Every 2nd month analysis was repeated. As the expiry period mentioned on bottles was one year, hence last analysis was completed in February 2006. For analysis, aluminum cover of each bottle was punctured with a sharp needle and 5 ml water was sucked with syringe for analysis. Bottles were again sealed with paper tape and left under ambient atmospheric conditions. Detailed results are given in Table 3 and average analyses of three bottles are plotted in Fig.1. Results indicate that there is significant increase of styrene with the passage of time. Increase in styrene and ethyl benzene was almost in the same ratio. Concentration of styrene was almost three times higher than WHO guide line values at the end of experimental period. There was no significant increase in benzene and toluene concentration. Apart from styrene and the other three quantified compounds, propyl benzene, 1ethyl 4-methyl benzene, α -methyl styrene, (1-methylethyl) benzene, 2-propenyl benzene, 1-ethyl 3-methyl benzene and *p*-xylene were also identified in some of the experiments, where the concentration of styrene was significantly higher. Presence of these compounds indicates that traces of these compounds were present as impurities of styrene

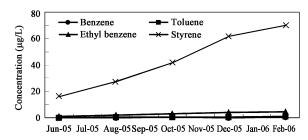


Fig. 1 Effect of storage time on leaching of styrene from PS bottles.

Table 1 Leaching of aromatic compounds in water from PS bottles (μ g/L)

| Brand | Packing date | Date of analysis | Benzene | Toluene | Ethyl benzene | Styrene | Total |
|---------|--------------|------------------|---------|---------|---------------|---------|-------|
| Brand 1 | October 04 | 31/1/05 | 0.16 | 0.14 | 1.58 | 20.35 | 22.22 |
| Brand 1 | October 04 | 31/1/05 | 0.22 | 0.18 | 1.20 | 20.05 | 21.64 |
| Brand 1 | May 05 | 9/7/05 | 0.13 | 0.21 | 1.77 | 16.00 | 18.11 |
| Brand 2 | April 04 | 31/1/05 | 0.80 | 0.41 | 0.76 | 16.28 | 18.24 |
| Brand 2 | May 04 | 9/7/05 | 0.13 | 0.21 | 1.77 | 20.05 | 22.16 |
| Brand 2 | April 05 | 12/7/05 | 0.13 | 0.20 | 1.60 | 15.84 | 17.77 |
| Brand 3 | June 04 | 31/1/05 | 0.16 | 0.19 | 3.21 | 29.50 | 33.06 |
| Brand 3 | October 04 | 31/1/05 | 0.73 | 0.28 | 2.46 | 26.96 | 30.42 |
| Brand 3 | June 05 | 9/7/05 | 0.16 | 0.04 | 0.42 | 12.29 | 12.90 |

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| Bottle | Date of analysis | Benzene | Toluene | Ethyl benzene | Styrene | Total |
|----------|------------------|---------|---------|---------------|---------|-------|
| Bottle 1 | 6/9/2005 | 0.13 | 0.20 | 1.60 | 15.84 | 17.77 |
| Bottle 2 | 6/9/2005 | 0.18 | 0.251 | 0.85 | 17.28 | 18.56 |
| Bottle 3 | 6/9/2005 | 0.14 | 0.28 | 1.08 | 15.45 | 16.95 |
| Bottle 1 | 8/9/2005 | 0.25 | 0.45 | 2.15 | 25.87 | 28.72 |
| Bottle 2 | 8/9/2005 | 0.18 | 0.51 | 1.95 | 27.45 | 30.09 |
| Bottle 3 | 8/9/2005 | 0.31 | 0.39 | 2.01 | 28.56 | 31.27 |
| Bottle 1 | 10/7/2005 | 0.25 | 0.44 | 3.15 | 42.25 | 46.09 |
| Bottle 2 | 10/7/2005 | 0.41 | 0.39 | 3.08 | 40.42 | 44.30 |
| Bottle 3 | 10/7/2005 | 0.38 | 0.64 | 2.98 | 42.25 | 46.25 |
| Bottle 1 | 12/8/2005 | 0.28 | 0.45 | 4.29 | 59.25 | 64.27 |
| Bottle 2 | 12/8/2005 | 0.24 | 0.68 | 4.15 | 62.31 | 67.38 |
| Bottle 3 | 12/8/2005 | 0.19 | 0.71 | 4.07 | 64.15 | 69.12 |
| Bottle 1 | 2/8/2006 | 0.28 | 1.24 | 4.54 | 69.25 | 75.31 |
| Bottle 2 | 2/8/2006 | 0.26 | 0.78 | 4.68 | 68.14 | 73.86 |
| Bottle 3 | 2/8/2006 | 0.41 | 0.61 | 5.06 | 71.21 | 77.29 |

in PS or by aging of PS, styrene may be degraded in to these by-products.

2.2 Styrofoam cups

Styrofoam cups, which had been examined for styrene monomer content, are used locally for tea and coffee. Hence it is very important to study the leaching capacity of these materials. In this study each Styrofoam cup (250-ml) was filled with 200 ml of either hot or cold water, capped or left open and allowed to stand for 60 min. The water was then analyzed for styrene monomer and other aromatic compounds. These cups are generally used for hot drinks, hence it was also important to investigate the effect of temperature on styrene leaching. For this study Styrofoam cups were filled with 200 ml potable water at 50, 70, 90 and 100°C and covered properly. After 60 min water from each cup was analyzed. The results (Table 4) show that at 50°C there was no leaching of any chemical in water. At 70°C only traces of styrene and ethyl benzene were present (1.16 and 0.57 μ g/L). At 90°C almost all the four components were identified and their total concentration was increased to 11.49 μ g/L. The same increasing trend was noted at 100°C as it is clearly shown in chromatograms (Fig.2). Total concentration of all the four compounds was increased to 21.68 μ g/L at 100°C (Fig.3). These results indicate that temperature plays a major role in the leaching of styrene and other aromatic compounds.

Different experiments were conducted to study the leaching of styrene and other aromatic compounds. In all these experiments boiling water was added in the Styrofoam cups and kept at ambient temperature for 60

Table 4 Temperature effect on leaching of aromatic compounds in Styrofoam cups ($\mu g/L$)

| Temperature (°C) | Benzene | Toluene | Ethyl benzene | Styrene | Total |
|------------------|---------|---------|---------------|---------|-------|
| Ambient | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 70 | 0.00 | 0.00 | 0.57 | 1.16 | 1.73 |
| 90 | 0.06 | 0.13 | 1.95 | 9.35 | 11.49 |
| 100 | 0.06 | 0.21 | 4.05 | 17.37 | 21.68 |

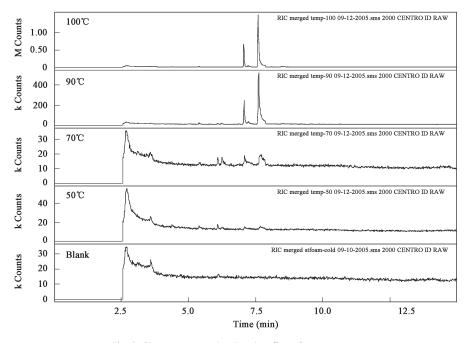


Fig. 2 Chromatograms showing the effect of temperature.

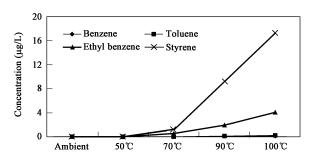


Fig. 3 Effect of temperature on leaching of styrene from styrofoam cups.

min. The purpose was to maintain the same atmospheric conditions in which these cups are used in daily life. There was a significant difference in the results from the cups kept open and closed (Fig.4). Obviously it is clear that from hot water in open cups, styrene and other aromatic compounds easily escaped from the surface due to high vapor pressure and low solubility in water. It is also reported that styrene released to surface water is rapidly lost through volatilization (EU, 2002). In closed cup volatilization loses were much less, hence styrene and other aromatic compounds concentration was higher. Similarly contents of styrene monomers also varied in cups from different manufacturer (Table 5, Fig.5). This may be due to the monomer contents present in PS. In some of the results concentration of styrene was much higher than WHO guideline value (Table 2). Concentration of other identified aromatic compounds was always less than WHO limits. Apart from above quantified four compounds the other identified compounds were: propyl benzene, 1ethyl 4-methyl benzene, α -methyl styrene, (1-methylethyl) benzene, 2-propenyl benzene, 1-ethyl 3-methyl benzene and *p*-xylene.

The quality of styrofoam cups is also important in leaching of styrene monomer. During these studies Styrofoam cups from two different manufacturer were purchased from super market and were labeled as "A" and "B". Results show that (Table 5) on average in "A" the styrene concentration (17.69 μ g/L) was much higher than in "B" (8.85 μ g/L). The concentration of other three compounds

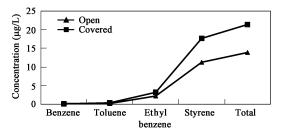


Fig. 4 Styrene leaching from open and covered styrofoam cups.

Table 5 Leaching of aromatic compounds from different materials $$(\mu g/L)$$

| Cups type | Benzene | Toluene | Ethyl benzene | Styrene | Total |
|-------------|---------|---------|---------------|---------|-------|
| Styrofoam A | 0.13 | 0.38 | 3.21 | 17.69 | 21.41 |
| Styrofoam B | 0.00 | 0.17 | 2.55 | 8.85 | 11.57 |
| PS cups | 0.00 | 0.06 | 0.13 | 2.03 | 2.21 |
| Paper cups | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

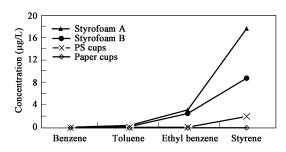


Fig. 5 Leaching of aromatic compounds from different materials.

was also slightly higher in "A". This may also be due to manufacturing defect and polymerization efficiency of the polymer. In "A" the unbound monomer may be more than in "B" and the same have been leached in the water. It may also be due to the aging of the styrofoam, storage conditions and manufacturing process. As these Styrofoam cups were purchased from local market, hence it can only be concluded that in "A" unbound monomer were higher than in "B". The same has been reported in literature that styrene monomer contents vary in different brands of PS (EU, 2002).

Small PS cups (80 ml capacity) locally used for tea and coffee were also studied for leaching of styrene monomer. In these cups 70 ml boiling water was added and analyzed after 60 min. From PS cups styrene monomers also migrated in hot water. The concentration of all the identified components was much less than in styrofoam. Paper cups (250 ml capacity) were also used in these studies. Both hot and cold water (200 ml) was added in paper cups and analyzed after 60 min. Even traces of organic volatile compounds were not detected in both hot and cold water. This indicates that paper cups are safe for hot drinks. These results show that leaching capacity of styrofoam cups is much higher than PS cups. In Styrofoam cups styrene leached more than WHO maximum allowable limit.

In almost all type of PS material, styrene monomer and some related aromatic compounds were leached in water. It may not have immediate effect but chronic effects may be observed as a result of repeated ingestion of a number of small doses, each in itself insufficient to cause an immediate acute reaction but in the long term having a cumulative toxic effect. Other three aromatics compounds are also highly toxic, but their concentration is much less than WHO guideline values. Chances are there that styrene might have synergetic effect with other aromatic compounds as it has been stated that in combination with other commonly used products, the toxicity of the migratory chemicals from plastic can be potentiated by synergy (Paul, 2003). Thus this and other similar chemicals have introduced a problem of protracted action of low concentrations upon human health. It is true that plastic ingredients do not act like pesticides (or a variety of other highly bioactive substances), and one can hardly expect immediate and pronounced clinical manifestations of their toxic action. The occurrence of acute toxicity due to plastic used in contact with food and drinking water is most unlikely, since only trace quantities of toxic substances are likely to migrate. However, it would be a great underestimation to consider plastic ingredients (indirect food additives) as presenting no real public health threat. The timing of the exposure can be much more relevant than its dose. Most vulnerable times are in periods of rapid growth, such as those in embryo and children right up to puberty (Paul, 2003).

3 Conclusions

Considering the toxic characteristic of styrene and leaching in water and other products, PS material should be avoided for food packaging. Especially PS rigid and foam cups should not be used for hot drinks. Paper cups are safe for hot drinks and have no threat as from PS cups. It is also recommended that public awareness program shall be launched to avoid Styrofoam cups for hot drinks.

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