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# Implications of Physico-chemical Factors on the Migration of Phthalate Esters from Tubing Commonly Used for Oral/Nasal Feeding

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There has been tremendous increase in production and consumption of plastics and polymeric materials during the past two decades. Due to their wide spread use, scientists have given attention for their toxicological evaluation and safety. Although, the finished plastic products are considered to be safe, biologically inert and are not suspected for any chemical leaching from them, if manufactured under standard test conditions. However, it has been reported that several chemical components of the plastic viz. unreacted monomers, plasticizers, stabilizers, heavy metals, u.v. absorbers etc., most of them toxic, leach out into the stored material (Alam et al., 1988, Jaeger and Rubin, 1970, 1972,, Wildbrett, 1973, Crompton, 1979) and put the consumer to the risk of health hazards. Phthalate esters are being extensively used in fabrication of medical and food grade plastics to impart flexibility. Phthalate esters have been reported to be hepatotoxic, teratogenic, mutagenic and carcinogenic in experimental animals (Seth, 1982, Tomita et al 1982, Autian, 1982). Studies conducted in our laboratory and elsewhere, have shown the migration of DEHP from plastic bags into edible oils, blood and life saving fluids (Dextrose and N-saline etc.) (Srivastava et al 1985, Jaeger and Rubin, 1972, Figge 1972). So far limited information is available on the extent of migration of phthalate esters from medical or food grade plastics under the conditions of their use. The present study aims at ascertaining the leaching of dimethyl phthalate (DMP), dibutyl phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) from the tubings, used in hospitals for oral/nasal feeding to seriously sick patients. During the course of its use liquid food of varying temperature juices and drugs which may be acidic, alkaline or alcoholic are passed through them for different time intervals. This prompted us to study the effect of temperature, duration and nature of extractants on the leaching behaviour of the commonly used phthalate plasticizers.

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## MATERIALS AND METHODS

The tubings free from paints and adhesive were cut into 5 x 0.3 cm surface area strips. The strips were washed thoroughly with tap water and then twice with distilled water, dried and weighed. 1 $cm^2$ surface area strips were extracted with 1 ml of extractant. The various extracting media and temperatures adopted for the extraction were based on the recommendations of British Pharmacopoea, British Plastic Federation, National Formulary, U.S.A., Bureau of Indian Standards and duly modified at Industrial Toxicology Research Centre for the safety evaluation of plastics used for packaging of food and medicines.

### Extractants

1. Distilled water
2. 8% Ethanol
3. 3% Acetic acid
4. 5% Sodium carbonate

### Extracting conditions

1. 70°C for 24 hours
2. 60°C for 1, 2, 5, 10 and 15 days
3. 40°C for 24 hours
4. ( 25°C) for 24 hours (Room temperature).

The total contents of phthalate esters were extracted from the tubing by refluxing the sample pieces with methanol:n-hexane (1:1) for 5-6 hours. The solvent was evaporated and residue was made up to 10 ml by methanol. DEHP, DBP and DMP from the various plastic extracts were extracted by the method of Poole and Wibberely (1977) with some modifications and quantified on HPLC. The extractants were extracted with acetonitrile and n-hexane twice. The organic phase of each step was mixed and evaporated, and made upto 10 ml with methanol. The sample thus prepared was filtered and injected in HPLC machine for identification. Each sample was analysed in quadriplicate.

HPLC varian Vista model (5500) with u.v. 20, Vista Ultra absorbance detector at 234 nm wave length, micropak 4 mm x 15 cm reversed phase column and 90:10 (methanol:water) mobile phase, was used. 10 ul of the sample was injected and the peaks obtained were compared with standard peaks obtained at that particular retention time. Results were quantified with a spectraphysics SP 4270 integrator and calculated as microgram of plasticizer migrated in 200 ml extractant.

## RESULTS AND DISCUSSION

The total contents of DEHP, DBP and DMP in tubing were found to be 225 mg/g, 54 mg/g and 28 mg/g respectively. The study on the migratory aptitude of phthalate esters from tubing revealed that the pattern of leaching of these plasticizers in general, was in the order of 5% sodium carbonate > 3% acetic acid > 8% ethanol > distilled water (Table 1-4). At 70°C, 60°C, 40°C and at room temperature (25°C) leaching of DEHP, DBP and DMP occurred to a maximum extent in 5% sodium carbonate. In 3% acetic acid leaching was less than 5% sodium carbonate, but higher than 8% ethanol and distilled water. Minimum leaching was seen in distilled water at all the temperatures and durations studied. The process of leaching was slow at room temperature (25°C) but as the temperature increased to 40°C, 60°C and 70° C, it also increased consistantly (Table 1). When the tubings were extracted in various extractants at 60 °C for different durations, the migration of DEHP, DBP and DMP was minimum on first day but it increased with the increase in duration of contact i.e. 2, 5 and 10 days significantly. The process of leaching slowed down after 10th day of extraction as there was no significant difference in the quantities of DEHP and DBP migrated on 10th day and 15th day (Table 2-4)

Our results indicate that enhanced leaching of phthalate esters may occur due to longer duration of contact and at an elevated temperatures. Chemical nature of extractants also played an important role on the leaching pattern. In plastics, plasticizer and polymer chain is bonded in such a way that former is embeded in the gaps of latter having the Wander Vaal's attracting forces with each other. But the leaching occurs due to the fact that the plasticizer components, which remain unreacted on the surface or the matrix of polymer, leach out in the commodities which passes through them. The results of our studies suggest that leaching of phthalate esters is a time, temperature and nature of extractant (acidity/alkalinity) dependent phenomenon.

The result of this study also manifests that varying quantities of phthalate esters may enter into the biological system of patients, while taking liquid food or drug through polymer tubings. Although the quantities of DEHP, DBP and DMP leched out were low in comparison to admissible daily intake for human beings (1 mg/kg body weight/day). However, other migrants of plastic or the small quantities of environmental pollutants viz. pesticides, fungicides, polynuclear aromatic hydrocarbons, heavy metals etc. present in biological system may alter the toxic response of even smaller

Table 1. Influence of temperature on the migration of DEHP, DBP and DMP (ug/200 ml) from tubing in various extractants kept for one day

| Extractants | 25 °C Room Temperature | | | 40 °C Room Temperature | | | 70 °C Room Temperature | | |
|---|---|---|---|---|---|---|---|---|---|
| | DEHP | DBP | DMP | DEHP | DBP | DMP | DEHP | DBP | DMP |
| Distilled water | 49±2.5 | ND | ND | 142±5.3 | 56±2.9 | 21±1.2 | 253±6.2 | 143±5.6 | 42±2.0 |
| 8% Ethanol | 65±3.8* | 16±1.5 | ND | 167±4.7* | 67±2.6* | 36±2.8* | 386±6.2* | 212±6.6* | 51±2.1* |
| 3% Acetic acid | 76±6.9* | 20±1.2 | 12±0.9 | 194±5.6* | 76±2.8* | 40±2.4* | 470±9.0* | 219±5.0* | 69±3.3* |
| 5% sodium carbonate | 74±3.7* | 20±1.4 | ND | 204±5.7* | 79±4.1* | 51±3.0* | 503±12.7* | 225±8.9* | 66±4.0* |

All values are mean ± S.E. from five samples.
*P <0.05 in comparison to distilled water.
DMP = Dimethyl phthalate; DBP = Dibutyl phthalate; DEHP = Di(2-ethylhexyl)phthalate

Table 2. Effect of duration on the migration of di(2-ethylhexyl)phthalate (ug/200 ml) from tubings in various extractnts at 60°C

| Extraction duration | Distilled water | 8% Ethanol | 3% Acetic acid | 5% Sodium carbonate |
|---|---|---|---|---|
| 1 day | 223.8± 2.80 | 347.0± 4.43* | 403.4± 2.89* | 420.4± 4.30* |
| 2 days | 275.0± 2.98 | 414.8± 4.49* | 469.4± 2.18* | 483.6± 5.31* |
| 5 days | 315.2± 7.31 | 476.4± 4.82* | 547.2± 3.73* | 569.6± 3.58* |
| 10 days | 613.0± 3.79 | 773.2± 5.75* | 968.2± 3.83* | 985.2± 7.25* |
| 15 days | 625.4± 5.30 | 801.8± 5.80* | 974.4± 13.95* | 1008.6± 3.14* |

All values are mean ± S.E. from five samples.
*P < 0.05 in comparison to distilled water.

Table 3. Effect of duration on the migration of dibutyl phthalate (ug/200 ml) from tubings in various extractants at 60°C

| Extraction duration | Distilled water | 8% Ethanol | 3% Acetic acid | 5% Sodium carbonate |
|---|---|---|---|---|
| 1 day | 118.6± 3.89 | 174.8± 5.11* | 207.4± 5.59* | 211.4± 5.84* |
| 2 days | 138.8± 4.23 | 209.4± 3.58* | 225.8± 5.10* | 238.0± 4.72* |
| 5 days | 202.8± 4.42 | 295.2± 5.67* | 323.4± 7.20* | 334.2± 8.27* |
| 10 days | 319.6± 8.91 | 402.6± 5.99* | 427.4± 5.66* | 434.6± 6.87* |
| 15 days | 323.2± 7.23 | 421.2± 4.79* | 442.6± 4.37* | 451.8± 3.39* |

All values are mean ± S.E. from five samples.
*P < 0.05 in comparison to distilled water.

Table 4. Effect of duration on the migration of di methyl phthalate (ug/200 ml) from tubings in various extractants at 60 °C

| Extraction duration | Distilled water | 8% Ethanol | 3% Acetic acid | 5% Sodium carbonate |
|---|---|---|---|---|
| 1 day | 38.6± 3.05 | 45.8± 2.01 | 52.0± 2.88* | 50.6± 3.37* |
| 2 days | 51.4± 2.42 | 71.4± 3.42* | 78.4± 8.65* | 78.8± 3.05* |
| 5 days | 69.0± 3.00 | 125.8± 10.01* | 107.6± 4.70* | 111.6± 3.62* |
| 10 days | 103.4± 2.67 | 138.4± 2.71* | 153.8± 3.76* | 156.6± 5.42* |
| 15 days | 107± 3.12 | 148.2± 3.26* | 161.4± 3.78* | 169.8± 4.89* |

All values are mean ± S.E. from five samples.
*P < 0.05 in comparison to distilled water.

quantities of DEHP, DBP and DMP reaching the patients suffering from various diseases. These studies are of significance in view of the reported toxic effects of phthalate plasticizers.

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**REFERENCES**

Alam MS, Srivastava SP, Seth PK (1988) Factors influencing the leaching of heavy metals from plastic materials used in the packaging of food and biomedical devices. Ind J Environ Hlth 30: 131-141

Autian J (1982) Antifertility effects and dominant lethal assays for mutagenic effects of DEHP. Environ Hlth Perspect 45: 115-118

Crompton TR (1979) Additives migration from plastics into food. Pergaman Press, Great Britain.

Figge K (1972) Migration of additives from plastic films to edible oil and fat simulants. Food Cosmet Toxicol 10: 315-327.

Jaeger RJ, Rubin RJ (1972) Migration of phthalate plasticizer from polyvinylchloride PVC blood bags into stored human blood and its localization in human tissue. New Eng J Med 287: 1114-1118
Jaeger RJ, Rubin RJ (1970) Plasticizers from plastic devices: extraction metabolism and accumulation by biological systems. Science 170: 460-462
Poole CF, Wibberely DG (1977) Chromatography Determination of DEHP in human placenta. J Chromat 132: 511-518
Seth PK (1982) Hepatic effects of phthalate esters. Environ Hlth Perspect 45: 27-34
Srivastava SP, Arvind K Saxena, Prahlad K Seth (1985) Migration of of di(2-ethylhexyl)phthalate (DEHP) from plastic containers used for packaging of Infusion fluids. Ind J Pharm Sci 47: 156-158
Tomia IY, Nakamura N, Aoki N Inice (1982) Mutagenic/ carcinogenic potential of DEHP and MEHP Environ Hlth Perspect 45: 119-125
Wildbrett G (1972) Diffusion of phthalic acid esters from PVC milk tubings. Environ Hlth Perspect 3: 29-35.

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