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Time Serial Concentration of Phthalate Esters and Bisphenol-A Contaminated from Spring Water Container's Cap and Seal Film

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Abstract : Industrial plasticizers such as phthalates can induce peroxisome proliferation. A growing concern among environmental and health groups has arisen because phthalates such as di-2-ethylhexyl phthalate (DEHP) and DBP may cause hormonal disorders, reproductive toxicity, hepatocellular tumors, genital disorders owing to a capacity to bind estrogen receptors, and a low-dose toxic action during certain periods of fetal development. Phthalate esters are used extensively as a plasticizer for plastic manufacture such as PVC bags and medical devices. This study investigated the effects of leached components from spring water container's cap and seal film. Phthalates, e.g. dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzylbutyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), and bisphenol A (BPA) were measured in the spring water. The bisphenol A was not detected or below the detection limit on the leaching from cap, sealing or spring water. DEHP were detected 90-116 ppb on the leaching from seal after 2 weeks, and 0.48-0.51 ppb from the spring water after 1 week. BBP were measured from seal within 1 week 25.4-66 ppb and below 0.12 ppb from spring water within 2 days. DMP were detected from seal within 2 weeks 51-68.5 ppb and 0.12 ppb after 2 weeks. DEP were measured from seal within 2 weeks 48.1-141 ppb and the concentrations were increased by the time from 0.10 to 0.31 ppb at spring water. DBP were detected from the seal within 2 weeks 92.3-5100 ppb and the concentration were decreased by the time from 0.24 to 0.10 ppb at spring water. These results indicate that some phthalate esters contaminated with spring water using the intact cap and seal film. It is concluded that the measured levels of phthalates leaching from these materials might *in vivo* only be slightly less than 1/10 of the LOAEL.

Keywords : phthalate esters, bisphenol A, spring water, cap, seal film, DEHP, DBP, DMP, BBP, DEP, LC-MSD

Introduction

Spring water is one of the drinking water that is managing with tap water and purified water by the Ministry of Environment. The consumptions of spring water have been increased rapidly because the economic enlargement made the public move to thinking of health. Therefore, the annual amount of container consumption is substantial from the worldwide.

PET (polyethylene terephthalate) container that has used to current spring water container is using worldwide by various advantages. The PET container manufactured by raw materials such as ethylene, styrene, ethyl glycol and bisphenol-A that extracted from crude oil. The phthalate esters used as a additives were classified from WWF (8 classes), Japan Ministry of Health, Labour and Welfare (9 classes), EPA (9 classes) as endocrine disrupter. Dioctylphthalate(DOP) was known representative materials. Negative effects from dimethyl phthalate (DMP), diethyl phthalate(DEP), dibutyl phthalate (DBP) and butyl benzyl phthalate(BBP) were been reported. Reproductive toxicity of di 2-ethylhexyl phthalate(DEHP) has been reported.[1)]

Appeared by the result BBP > DBP > DEP that was from using microorganism tests for measuring phthalate's estrogenicity. In the case of BBP, the estrogenic potentials compared with 17β-estradiol was 10^{-6}~10^{-7} that was proved by estrogen α-receptor binding assay. DEHP was the material known as toxic effect to human among phthalates. MEHP and 2-EHA, metabolites from DEHP were related to inducing reproductive toxicity and hepatocellular tumors. Reproductive toxicity by run out the zinc ion from testes has been reported.[2,3)]

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Bisphenol-A have been known as a modulaotr of cell cycle, as a inducer of DNA damage, as a estrogen mimics and as a chromosomal abberation agents by telomeric association. Especially bisphenol-A that was used in dentistry and food preservatives was known very high of estrogenicity from experiments on animals and breast cancer cells. This chemical could induced estrogenicity by binding to α and β-receptor.[4,5]

These materials are leaching to spring water from the cap or seal film. Some reports have been commented the possibility to come from. Even though these materials leaching from container's cap or seal itself, it could not avoid contaminating. The are some cases such that do not remove spring water container's cap or seal film from the container as it is, and how to handle the drilling pin make it possibe expose the toxic substance from leaching.[6,7]

This study carried out to find out that phthalate esters and bisphenol A quantity leaching from spring water container's cap or seal film did it exist at spring water according to leaching time in the water or it's conditions.

Materials and Methods

Analysis Material

Analysis device that was used to analyze phthalate esters and bisphenol-A connects used Waters 2690 HPLC auto injectors and controller to LC/MSD that attach Waters 2690 HPLCs (Waters Inc.) and Mass Selective ZQ 2000 (Micro Mass Inc.) by series. LC/MSD used in this study were applied that phthalate analysis of LC/MS-ESI positive ion mode, and bisphenol-A analysis of LC/MS-ESI negative ion mode. Nitrogen concentrator used for concentrating of sample were N-EVAPRTM 112 (Organomation Associactor Inc.), and purity of nitrogen gas used to prevent pollution at concentration process used more than 99.999%. Standard reagent(Ultra Scientific Inc, USA) was more than 99% purity, and HLB (Hypo-philic Lipophilic Balance, 6cc 500 mg, Waters) cartridge was used in concentrating and purifing the samples.[6,9] And also, all glass-wares which were used to experiment were washed by automatic washer (NEWMACTIC LA2). This machine operated washing and drying more than two times, after then the cleaned glass ware preserved at clean space seal off by aluminum foil, and we used it washing by solution at analysis. All reagents and extraction materials used in the other experiments were used high purity to exclude the possible measurement error by pollution or impurities.

Sample Pretreatment

Analytical samples were collected to seruak time schedule 1 week, 2 weeks and 3 weeks in 30 m*l* distilled water that was socked by container's cap 1 ea (7.5 g/ea) and seal film 1 sheet (1.1 g/sheet). That cap or films used were the same as the spring water in 17 *l* spring water container. Experimental temperature of samples were 4°C as a spring water temperature stored at usual condition. Blank was that the identical sample was pretreated for cap or seal not contact with spring water.

Bisphenol A was conditioned by MTBE for HLB cartridge at pH3. The cartridge was washed to methanol and distilled water after then the sample was injected. This procedures were used that methanol 5% for washing, nitrogen gas by MTBE contain methanol 10%, and 1 m*l* acetonitrile. Phthalate conditioning by MTBE for HLB cartridge was carried out at pH3, after washing of methanol and distilled water, the sample was injected.[10,11]

Instrumental Analysis

To find out the ion spectrum of DMP we made compose the user library for qualitative confirmation cone voltage changed step from 10V to 50V. The primary ion m/z 195$[M+H]^+$ was high intensity at 15V, after then it was decreased as the cone voltage was increasing. The fragment ion of DMP, m/z 163 was trace at below 15V, as the voltage was increased this ion potential was increase to 30V. Both of them were decreased at high cone voltage above 40V. From these results, we set out the rate of fragment ion/ parent ion, cone voltage was 20V for qualitification. Fragmention/parent ion at 20V was 100 : 72 constantly even though the mz 163/ mz 195 was changed. We used this rate as user library. Another method to qualitification was to using isotopcal MS or HR/MS. We applied this method as a secondary qualitification method. The theoretical isotope rate of dimethyl phthalate was m/z 195 :

Table 1. The LC/MS operating conditions for determination of phthalates

	Activity	Condition
LC	column	X terra™ MS C_{18} 3.5 μl 2.1 × 150 mm
	mobile phase	A: Water B: Acetonitrile C: 0.5% acetic acid
	gradient	50% B linear to 90% B in 10 min
	flow	0.2 ml/min
	stop time	32 min
	column temperature	30°C
	sample temperature	20°C
MS	type	SIR
	mass	195(dimethyl phthalate), 223(diethyl phthalate), 279(dibutyl phthalate), 313(butyl benzyl phthalate)
	ion mode	ES+
	cone(V)	15V(dimethyl phthalate, diethyl phthalate, dibutyl phthalate, butyl benzyl phthalate)
	source temperature	120°C
	desolvation temp.	250°C
	cone gas flow	65°C/hr
	desolvation gas flow	267 l/hr
	LM1 resolution	15.0
	HM1 resolution	15.0
	mutiplier	650
	run time	32 min

m/z 196 = 100 : 11.42, and the standard rate of isotope was 100 : 11.47 (IDL), and the rate of isotope same as pretreated mentioned above was 100 : 10.80 (MDL). We used this rate of isotope as a confirmational user library. Column that use phthalate and bisphenol-A in separation used Waters company's XTerra™ MS C_{18} column (2.1×150 mm, 3.5 μl). Distilled water, aceonitrile and 0.5% acetic acid used as a mobile phase. Detailed instrumental condition used in analysis presented to Table 1 and Table 2.

Results and Discussion

The samples were analyzed according to passage time that was spring water container's cap (7.5 g/ea)

Table 2. The LC/MS operating conditions for determination of bisphenol A

	Activity	Condition
LC	column	X terra™ MS C_{18} 3.5 μl 2.1 × 150 mm
	solvent ratio	A: Water(40) B: Acetonitrile(50) C: 0.5% acetic acid(10)
	flow	0.2 ml/min
	stop time	15 min
	column temperature	30°C
	sample temperature	20°C
MS	type	SIR
	mass	227
	ion mode	ES-
	cone(V)	40V
	source temperature	120°C
	desolvation temp.	250°C
	cone gas flow	64 l/hr
	desolvation gas flow	267 l/hr
	LM1 resolution	14.5
	HM1 resolution	16.1
	mutiplier	650
	run time	15 min

Table 3. The concentrations of bisphenol A, DEHP and BBP measured in the water soaked by cap and seal

Parts	Time	Bisphenol A	DEHP	BBP
Seal	1 day	nd	nd	25.4
	1 week	nd	nd	66
	2 weeks	nd	90	nd
	3 weeks	nd	116	nd
Cap	1 day	nd	4.2	nd
	1 week	nd	11.1	7.1
	2 week	nd	18.5	na
	3 week	nd	18.4	na

Table 4. The concentrations of DMP, DEP, and DBP measured in the water soaked by cap and seal

Parts	Time	DMP	DEP	DBP
Seal	1 day	nd	48.1	92.3
	1 week	51	127	5,100
	2 weeks	68.5	141	1,201
	3 weeks	nd	nd	nd
Cap	1 day	2.3	6.4	3.7
	1 week	nd	2.2	140
	2 week	5.3	4.8	76.5
	3 week	na	na	na

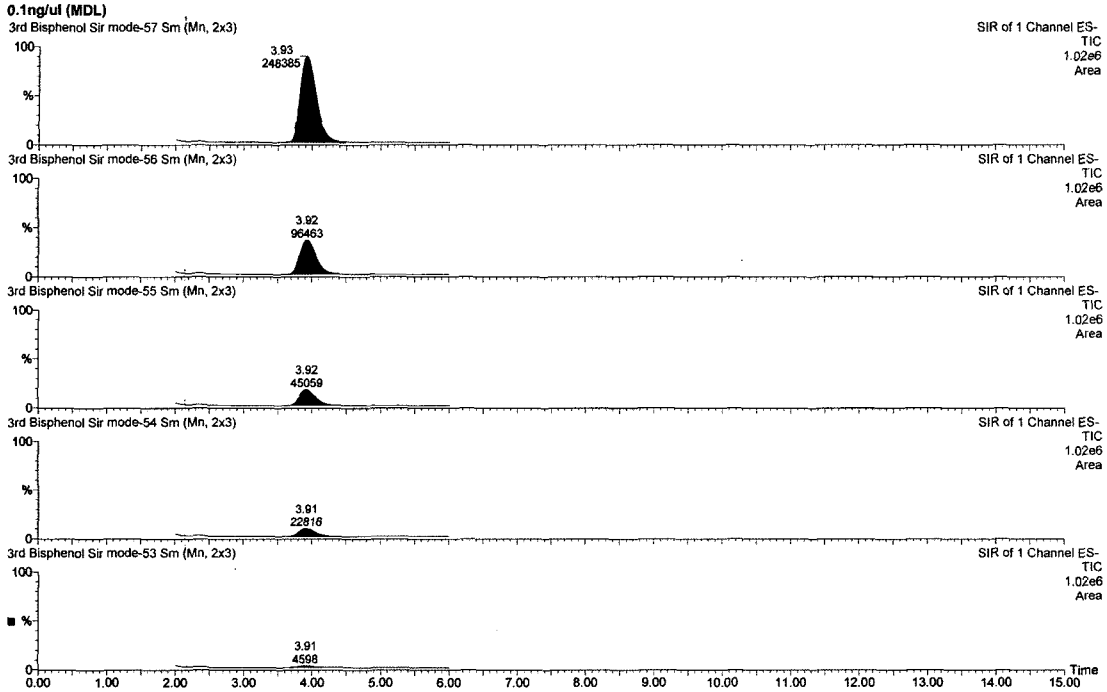

Fig. 1. Ion chromatogram of bisphenol A at m/z 227 [M-H]+ according to concentration.

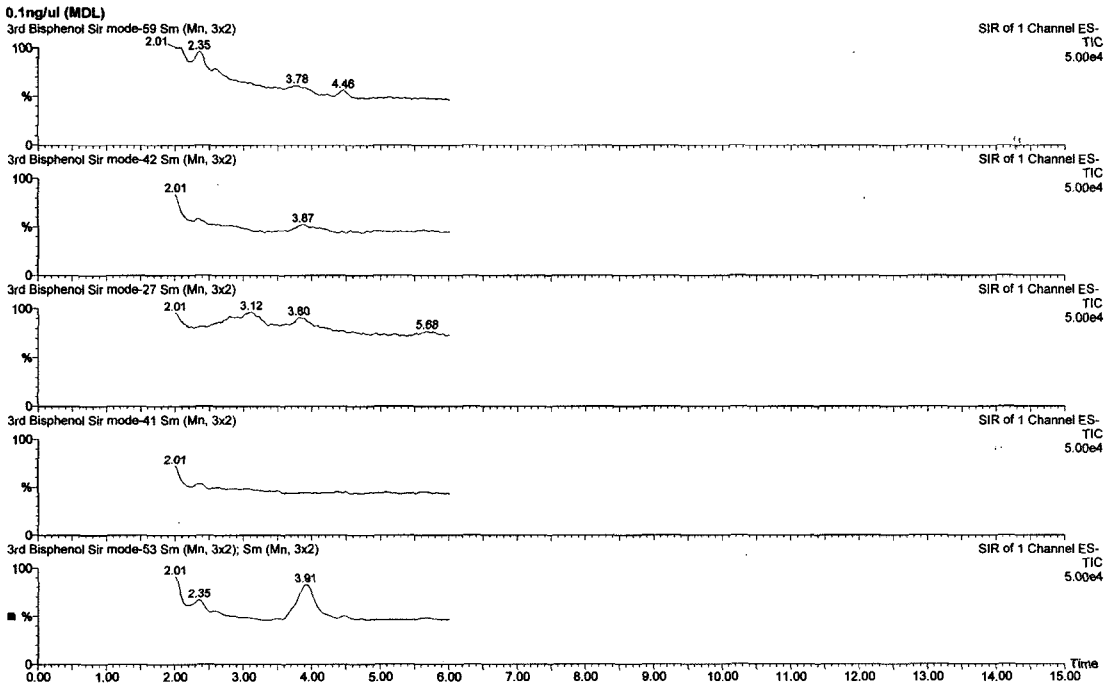

Fig. 2. Ion chromatogram of bisphenol A of sample (SIR mode).

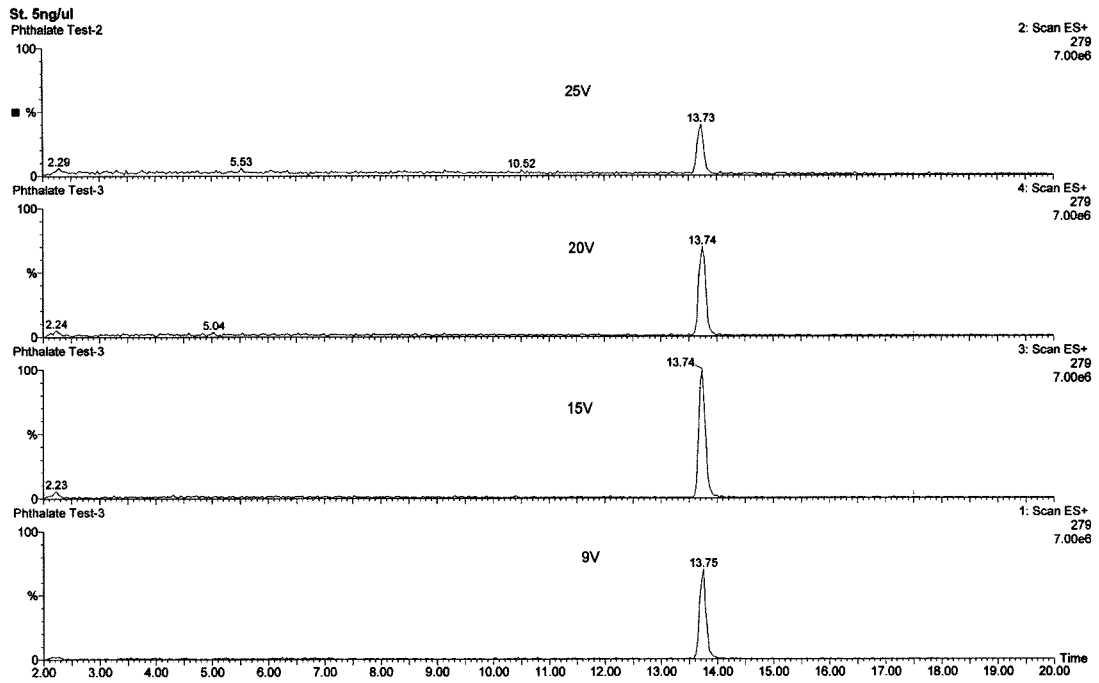

Fig. 3. Ion chromatogram of DBP (dibutyl phthalate) according to cone voltage.

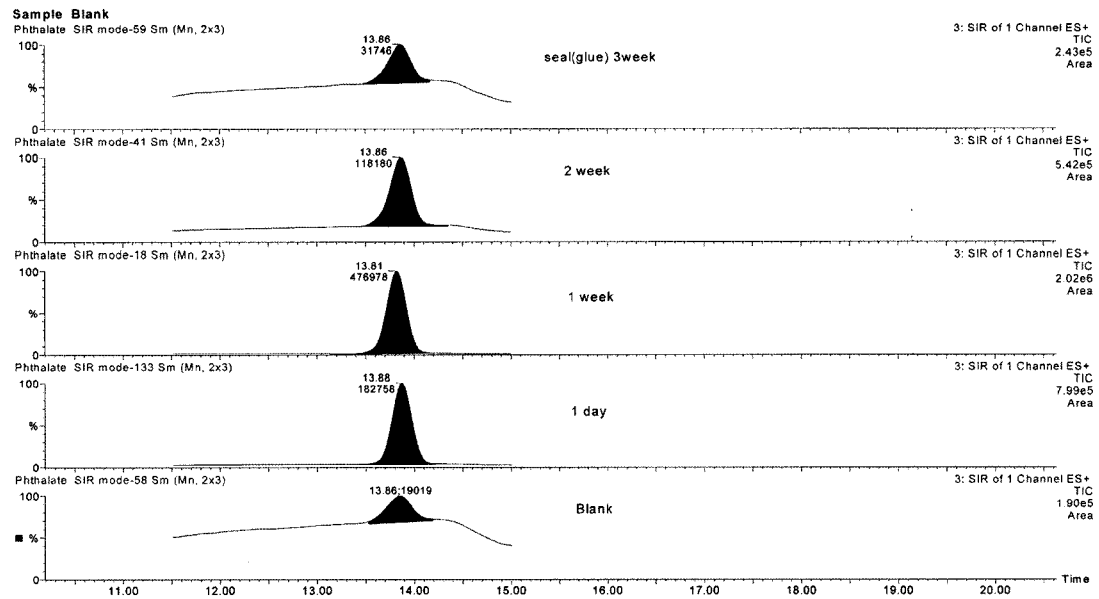

Fig. 4. Ion chromatogram of DBP (dibutyl phthalate) from seal by time.

or seal film (1.1 g/sheet) soaked each in distilled water (about 30 m*l*). Spring water container's seal film used in analysis sampling after 1 day, 1 week, 2 weeks, and 3 weeks. Spring water container's cap used in analysis sampling after 1 day, 1 week, and chromatogram of analysis figure from 1 to 5,

Table 5. The concentrations of bisphenol A, DEHP and BBP measured in the water conventionally used spring water system

Parts	Time	Bisphenol A		DEHP		BBP	
Hot/Cold		Cold	Hot	Cold	Hot	Cold	Hot
	3 hrs	nd	nd	nd	nd	0.08	0.08
	24hrs	nd	nd	nd	na	0.10	0.09
water	48 hrs	nd	nd	nd	na	0.11	0.12
	1 week	nd	nd	0.51	0.52	na	na
	2 weeks	nd	nd	0.48	0.55	nd	nd

Table 6. The concentrations of DMP, DEP, and DBP measured in the water conventionally used spring water system

Parts	Time	DMP		DEP		DBP	
Hot/Cold		Cold	Hot	Cold	Hot	Cold	Hot
	3 hrs	Nd	Nd	0.10	0.17	Na	0.18
	24hrs	nd	nd	0.20	0.19	0.24	0.19
water	48 hrs	nd	nd	0.18	0.21	0.18	0.22
	1 week	na	na	0.21	0.26	0.11	0.10
	2 weeks	0.12	0.14	0.25	0.31	na	na

result appeared Table 3 and Table 4.

Analyzed 200 m*l* each cold waters and hot water to space 3 hours, 1 day, 2 day, 1 week, 2 weeks and 3 weeks to grasp effect dissolved to spring water in spring water container which spring water cap and seal film are not removed. Also, handle spring water that was not exposed to spring water cap and seal film to grasp blank level equally with pretreatment process of sample and did this by blank sample.

Bisphenol A were not detected that were spring water container's cap or seal film in spring water.

DEHP were not detected from seal to 1 week, but 90 ppb were detected after 2 weeks, and 116 ppb were detected after 3 weeks. DEHP were detected from spring water container's cap that were 2 ppb after 1 day, 11.1 ppb after 1 week. While, DEHP concentration change that follow in passage time of spring water 0.51 ppb (hot water 0.52 ppb) were detected after 1 week, 0.48 ppb (hot water 0.55 ppb) were detected after 2 weeks. The most amounts of DEHP were detected later all with 1day after and 1 week in spring water container's cap, and were not detected after 1 week in seal of spring water container but more than 90 ppb were detected after 2 weeks since. 0.51 ppb of detection among spring water was confirmed after 1 week, and much amounts were detected in hot water than cold water. DEHP progress in container's cap and seal since 1 week and expressed special quality that extremely small quantities were detected from passage 1 week during spring water being detected after 1 day from container's cap.

BBP were detected 25.4 ppb after 1day from seal, and were increased by 66 ppb after 1 week but was not detected since. Container's cap was not detected at 1 day passage a point of time, but 7.1 ppb were detected at 1 week passage a point of time. This has shown special quality that BBP detection in container's seal and cap gets into maximum after passage 1 week. BBP were detected 0.08 ppb from 3 hours passage a point of time in spring water system, 0.10 ppb were detected after 24 hours and after 48 hours 0.11 ppb, but was not detected after 1 week. The results that was detection special quality of spring water that BBP were detected from spring water container's cap and seal, and not detected after 1 week after show maximum detection amount in 1 day, 2 days passage a point of time of special quality and spring water that show the maximum detection amount after 1 week shows to be different, therefore assumed that BBP were

detected in spring water was possibility that do not leaching in spring water container's cap and seal.

DMP was not detected in 3 weeks passage a point of time, but 51 ppb were detected at 1 week passage point of time and show the maximum detection amount by 68.5 ppb after 2 weeks from seal. But, 2.3 ppb were detected at 1 day passage a point of time in cap, but was not detected at 1 week passage a point of time. Therefore, DMP detection special quality in spring water container's seal and cap shown to be different. The results detected of spring water were 0.12 ppb at 2 weeks passage a point of time. Therefore, DMP were detected of spring water was considered that it appears after with 2 weeks that was leaching from seal that cap was not.

DEP were detected 48.1 ppb at 1 day passage a point of time from seal, and 127 ppb at 1 week passage a point of time, show maximum detection from 2 weeks passage a point of time to 141 ppb and was not detected since. The other side, show results in cap that 6.4 ppb were detected at 1 day passage a point of time, and decreases from 1 week passage a point of time to 2.2 ppb. Spring water were detected 0.10 ppb from using 3 hours and increased unrelentingly to 0.25 ppb of passage a point of time 2 weeks since, and show tendency that the detection amount increases in hot water. DEP were detected of spring water was presmptive that it were detected that was leaching from seal.

DBP were detected 92.3 ppb at 1 day passage a point of time from seal, it has shown maximum from 1 week passage a point of time to 5,100 ppb and high level were detected from 2 weeks passage a point of time to 1,201 ppb, but was not detected after 3 weeks. DBP were detected at 1 day passage a point of time 3.7 ppb in cap, 1 week passage a point of time 140 ppb. Maximum were detected of spring water to 0.24 ppb at 24 hours a point of time, and decrease since 48 hours 0.18 ppb, 0.11 ppb were detected since 1 week, but was not detected after 2 weeks. The detection amount of DBP got into maximum from seal and cap at 1 week passage a point of time, but seeing tendency that decrease after passage 24 hours of spring water, could not exclude detection possibility by other pollution source that detection amount of spring water was not from seal or cap.

Endocrine disrupter has been known as chemicals that was styrene, ethylglycol, bisphenol-A, lead, cadmium and plasticizer phthalate. It has been known as representative plasticizer that give softness to phthalate esters PVC that increase produced by esterification reaction with alcohol coming phthalic anhydride. Dioctylphthalate(DOP) is representative material and dimethyl phthalate, diethyl phthalate, dibutyl phthalate, butyl benzyl phthalate. There were effect by BBP > DBP > DEP period of according to results that was most have reproductive toxicity, and measure each phthalate's estrogenicity using microorganism with these materials and that effect of 10^{-6}~10^{-7} was proved comparing 17β-estradiol in BBP.[14,15] Di(2-ethylhexyl) phthalate(DEHP) was PVC product, especially, plastics plasticizer used often to give softness to plastics medical treatment, and soft plastics to compose, there was case added 40% of plastics product raw material sometimes. Also, DEHP was used to yoghurt a bottle and milk pack of heating adhesion parts plastics and aluminum foil, and was used in ink, electric insulation, cosmetics ingredient, and agricultural chemicals, DEHP is dividing by to endocrine disrupter that doubt from WWF.[16]

Plastics connection endocrine disrupter has affinity with human's life such as many plastics receptacles used in current civilized society, syndet, lubrication oil and much problem was caused socially. Can classify by plasticizers and surfactants and synthesis connection material that intenerate plastic at process that make resin on this category or was used make in prescribed form.

Phthalate esters was used by plasticizer, 1/4 of total phthalate producing since 1930 years was diethylhexylphthalate(DEHP) and WWFs of phthalate classed by endocrine disrupter get into 8 kinds, Japan Ministry of Health, Labour and Welfare get into 9 kinds, and were detected in all plastics product, pack, vinyl a floor covered with laminated paper, emulsions paint, infant powdered milk, cheese, and margarine.[17] There was alkylphenols, alkyphenol ethoxylates, bisphenol-A, strene dimer and trimer with connection endocrine disrupter since surface active agent or composite number, and these was detected and exposed in nature environment through number of rivers and detect in fish or a bird or food along food chain in courage that make from wastewater or plastic outpoured in

home as syndet shampoo, surfactants of insecticide or weed killer, disjointing product of polycarbonate or polystyrene mainly.[17,18]

About 5% of crude oil was used by each kind petrochemistry product production raw material of ethylene, styrene, ethylglycol, bisphenol-A. Plastics (thermoset) that were used this raw material was produced divides by thermoplasticity plastic (thermoplastics) and thermosetting plastic(thermoset). Endocrine disrupter have been known as chemicals that is styrene, ethylglycol, bisphenol-A, lead, cadmium and plasticizer phthalate.

Bisphenol-A were detected in canned food that used was polycarbonate resin, and also was used by waterproof for dental surgery and composition materials.

Di(2-ethylhexyl) phthalate and di-n-butylphthalate of Phthalate esters by the most representative plasticizer was used. Phthalate esters because esters is insolubility as solubilization act transfer and flow in soil, sea and rivers that being found in state that accomplish fulvic acid components and complex that mediate, it has been known that was accumulated well in animal fat of living body. DBP lessen more than octylphenol of environmental estrogen but show estrogen property, and know that general effect of DBP is accumulation from truth that do not operation under endocrine estrogen's existence. It has been known DBP acts by reproduction and occurrence toxicant, and the effect does that is much seriouser in next generation than first generation. DEHP was fast metabolism from most variety to monoethylhexylphthalate(MEHP) and 2-ethylhexanoic acid (2-EHA). DEHP and MEHP and 2-EHA of metabolism connect with reproductive toxicity and hepatocarcinogenic propertiesact, partially operation by the genital gland toxicant that run dry zinc ion. DEHP was induces division of reproductive cell (spermatocytes and spermatids), though causes testes retrogression conducting disturbing transmission of nutritive substance that was extinction and destructive of reproductive cell from sertoli cell there are report of research that depend just in change of biological membrane by DEHP. Also DEHP has been known that cause hepatocarcinogenesis in liver poly(ADP-ribose) polymerase vitality derive. It has been known that effect to co-genotoxic that induce cutting of DNA duplex spiral structure by truth that DNA damage activity increases more derived by NDMA when exist with materials such as N-nitroso dimethylamine(NDMA).[2,3,19]

Conclusions

The analyzed concentrations by LC-MS for bisphenol-A and phthalate esters leaching at spring water container's (17L) cap or seal film were detected during spring water cap or seal film. The results were same as following.

1. Bisphenol A was not detected that were soaked spring water container's cap or seal film in spring water.

2. DEHP was detected in seal film at 2 weeks 90 ppb, 116 ppb at 3 weeks. Detected in spring water cap at 1 day 4.2 ppb, 11.1 ppb at 1 week. Detected in spring water at 1 week 0.51 ppb (hot water 0.52 ppb), 0.48 ppb (hot water 0.55 ppb) at 2 weeks.

3. BBP was detected in seal at 1 day 25.4 ppb, and was increased at 1 week by 66 ppb but was not detected after this time. 7.1 ppb were detected at 1 week in cap of container. The maximum detection amount BBP showed in seal or cap at 1 week. BBP in spring water were detected from at 3 hours after 0.08 ppb, 24 hours 0.10 ppb, 48 hours 0.11 ppb, but was not detected at 1 week.

4. DMP was detected from seal at 1 week 51 ppb, 68.5 ppb, at 2 weeks and the concentrations were showed maximum amount by 68.5 ppb but was not detected after then. However, the level of DMP was detected 2.3 ppb at 1 day in cap. The detection characteristics of seal and cap were something different. It were detected 0.12 ppb at 2 weeks passage a point of time in spring water. Therefore, DMP was detected most in spring water could presume that it appears at 2 weeks that was leached from seal but cap was not.

5. DEP were detected 48.1 ppb at 1 day from seal and 127 ppb at 1 week, was showed maximum detection 141 ppb at 2 weeks and was not detected since. The result showed that 6.4 ppb were detected at 1 day, and the concentration was decreased by 2.2 ppb at 1 week in cap. DEP were detected 0.10 ppb at 3 hours in spring water and was increased to 0.25 ppb at 2 weeks. The detection tendency was that the detection amount increases in hot water. DEP detected in spring water presumed that the

detection materails was from seal film.

6. DBP in seal at 1 day 92.3 ppb, expressed maximum by 5,100 ppb at 1 week and high level were detected by 1,201 ppb at 2 weeks. But DBP was not detected after 3 weeks. It was detected 3.7 ppb at 1 day, 140 ppb at 1 week from cap. The maximum level was detected by 0.24 ppb at 24 hours in spring water, and then the levels were decrease since 48 hours 0.18 ppb, 0.11 ppb at 1 week. But DBP was not detected after 2 weeks. The detection levels of DBP were maximum from seal and cap at 1 week passage a point of time. But the tendency of level such facts that decreasing at 24 hours in spring water, we could not exclude detection possibility by other pollution source from not seal or cap.

The level of phthalates in spring water which were leached from container's cap or seal film were not reached to the concentration level that the acute or chronic poisoning symptoms were inducing to human body. Phthalates that were leached spring water could not exclude possibility of pollution by others as a primary factor as well as cap or seal. But, these were classified as materials from EPA and WWF several institutions or the country to important endocrine disrupter currently. Therefore, it is need to let them know the fact that You should remove cap and seal at spring water container before it use. That is desirable because the adverse effect may expected to happen when use it over long term.

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