



## GENETIC EFFECT OF MIGRATORY COMPOUNDS FROM BOTTLED NATURAL DRINKING WATER STORED UNDER DIRECT SUNLIGHT

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### ABSTRACT

The drinking water is one of the important routes for human exposure to contaminants through releasing of antimony (Sb) and polymers from polyethylene terephthalate (PET) plastic. The aim of this study was to investigate the effect of sunlight on chemical compounds migration into PET-bottled water and studying the cytotoxicity of di-butyl phthalate (DBP) and di-octyl phthalate (DOP) by SMART in *Drosophila melanogaster*. Four random water bottle samples produced by various companies as: A (PET, clear, 0.6L), B (PET, clear, 1.5L), C (PET, blue, 1.5L) and D (polycarbonate PC, blue, 19L) for studying the effect of direct sunlight exposure on migration of antimony and phthalates. Experiment was carried out in the presence of sunlight (7 h daily) for 210 day. The migrated compounds profile ( $\mu\text{g/L}$ ) that detected sample (A) before storage were only two compounds formaldehyde (FA) and acetaldehyde (AA) out of nine compounds. During storage under sunlight, four compounds (Sb), bis-phenol A (BPA), dimethyl phthalate (DMP) and diethyl phthalate (DEP) were increased till 30th day then did not affect till the end of storage. Other detected compounds were continuously progressed till the end of storage with different rates. The highest rate was appeared in case of FA, it was 222 fold followed by DOP compound (173 fold) as well as DBP (75.3 fold) and finally the AA compound with 17.9 fold. Regarding to another sample B, only 3

compounds (BPA, DMP and DBP) were not detected at zero time. The AA compound was detected with the highest concentration ( $0.9\mu\text{g/L}$ ) and the lowest one was DOP ( $0.007\mu\text{g/L}$ ). Only four compounds were detected before storage named Sb, DOP, FA and AA, the lowest level ( $0.006\mu\text{g/L}$ ) was noticed in DOP, while the moderate level was recorded in FA ( $0.03\mu\text{g/L}$ ) and Sb ( $0.08\mu\text{g/L}$ ). Other detected compounds; i.e. DBP, DOP, AA and FA were consequently increased with higher levels. This study investigated genotoxic effects of (DBP) and (DOP) at  $30\mu\text{g/ml}$  concentration using the somatic mutation and recombination test (SMART). The cytotoxicity of the tested phthalate compounds was also assessed at five different concentrations 0.5, 1, 5, 10 and  $20\mu\text{g/ml}$  in two types of human cell lines; liver cancer (HepG2), colon cancer (HCT-116) using neutral red cytotoxicity assay. All of tested compounds significantly showed high levels of tumor induction and frequency compared to the negative control in SMART assay. It was also reduced the viability of the HepG2 cell lines cells using different concentrations and the highest cytotoxic effect. While, on HCT-116 showed no cytotoxic effect.

### INTRODUCTION

Bottled water is considered as an important source of clean water. Polyethylene terephthalate (PET) is the most popular material for food or beverage packaging (ILSI-Europe, 2000) and phthalate compounds are used as softeners and plasticizers in a wide range of plastic materials. PET bottles are occasionally exposed to direct sunlight due to mishandling in retail shops which

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causes leaching of some substances through thermo-mechanical process into the bottled water (Bach et al 2012). Antimony trioxide ( $Sb_2O_3$ ) is used as a catalyst in the manufacture of PET, so it can be leaching to water inside the bottle (Shotyk and Krachler, 2007). Formaldehyde, acetaldehyde, (BPA), (DMP), (DEP), (DBP), di (2-ethylhexyl) phthalate (DEHP) and (DOP) can be released as harmful chemicals from PET bottles into water. Chemically, phthalates are not bound to products, for this reason, it can easily leak out to enter the environment and diffuse within the materials due to their lipophilic properties (Fujii et al 2003; Kavlock et al 2006). Dibutyl phthalate (DBP) can pass through the placental and blood (Huang et al 2014) and found in rat brain (Williams and Blanchfield, 1975). Dioctyl phthalate (DOP) changed lymphoid organs in male rats, affected on cell viability of lymph nodes and increased the size and number of lysosomes and golgi apparatus (Dogra et al 1985).

There has been a growing concern regarding a possible health hazard to humans by migrated compounds (Kleinsasser et al 2000). Rapid, inexpensive and sensitive Somatic Mutations and Recombination Test (SMART) in *Drosophila melanogaster* is used successfully to detect carcinogenic compounds (Nepomuceno, 2015). Induction of tumors in *Drosophila* instead of marker clones might be directly adverse the risk of these migrants for inducing cancer in humans (Sidorov et al 2001). The application of mammalian cell cultures cytotoxicity assays for quantitating the potencies of cytotoxin agents included either the MTT colorimetric cell viability assay (Borenfreund and Puerner 1984) or neutral red cell viability assay (NR) (Fotakis and Timbrell 2006). The NR assay has been found to be more sensitive than the MTT assay (Hansen et al 1989).

*Drosophila imaginal* discs in larvae, (which develop into adult organs; i.e. wings, eyes, legs ... etc), are considered to be good material for genetic study at somatic cell level using suitable marker(s) in heterozygous condition allows to detect the genetic damage induced in the somatic cell led to loss of heterozygosity (LOH) that appears as mosaic spot on the corresponding cell organ.

In SMART systems, the frequencies of induced mutations depend on the expression of marker genes in different organ with regard to the temporal distribution of cell divisions in different developmental stage of the fly. Most of tumor suppressor genes are recessive lethals, clones homozygous of each genes induced in heterozygous lar-

vae manifest as tumors outgrowth in adult flies. However, both mutagen compounds were assayed using tumor mosaic spot test (*wts*) to evaluate and to compare the efficiency of these assays to detect potential genotoxicity hazards in *Drosophila* as a model system.

From the point of view that drinking water is one of the primary routes for human exposure to contaminants through releasing of Sb and polymers from PET plastic, the aim of this study was to investigate the effect of sunlight on chemical migration into PET-bottled water and studying the cytotoxicity of DBP and DOP by SMART in *Drosophila melanogaster*.

## MATERIALS AND METHODS

Standards of the individual phthalate esters: (DMP), (DEP), (DBP), (DOP) were purchased from a World of Fine Chemicals (CDH). Bisphenol A was purchased from Alpha Chemika (Mumbai). All reagents were analytical grade and checked for contamination with phthalates before using. Mycophenolic acid (MMC: C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>O<sub>5</sub>) manufactured by Bristol-Myers Squibb Caribbean Company Mayaguez, Puerto, USA was used. *Drosophila* stocks carries a recessive lethal *wts3-17* allele with the genetic structure of ♀ *st1* in *1* *Kni.ri-1* and *ppwts3-17/TM3.sb1* and ♂ wild type were provided by Bloomington *Drosophila* Stock Centre of the University of Indiana, USA, under registry No. Bloomington/ 7052 (Lindsely and Zimm 1992).

Four random water bottle samples produced by various companies as: A (PET, clear, 0.6L), B (PET, clear, 1.5L), C (PET, blue, 1.5L) and D (polycarbonate PC, blue, 19L) were taken from a local market in Cairo, Egypt for studying the effect of direct sunlight exposure on migration of antimony and phthalates. Experiment was carried out in the presence of sunlight (7 h daily) for 210 days (Muhammad et al 2011). Control sample was protected from direct sunlight exposure by storing in a dark place at room temperature (22-25°C).

Total Sb concentration was detected using hydride generation atomic fluorescence spectrometry (Carneado et al 2015). Formaldehyde was determined by Spectrophotometer (DR 2000) (Matthews and Howell, 1981). Acetaldehyde analyzed on a high performance liquid chromatography (Azra et al 2012). (DMP), (DEP), (DBP), (DOP), N, N-Diethylhydroxyl phthalate (DEHP) and (BPA) were determined by gas chromatograph (HPLC ultra, Flexar LC / Flexar FX-20, Perkin Elmer) as (Tienpont et al 2005).

### Somatic mutation and recombination tests (SMART)

The *wts/TM3* females were crossed to wild type males. After 2 days the parental flies were removed and 56-68 hours old larvae were transferred to a standard *Drosophila* medium containing 20 µg/ml of an appropriate Mitomycin C (MMC) solution for 24 hours, then transferred to standard *Drosophila* medium. For (DBP) and (DOP) treatments; 30 µg/ml solution of (DBP) and (DOP) were mixed in 100 ml of standard *Drosophila* medium at 50°C. All *Drosophila* stocks and crosses were maintained at 25±3°C. To score *warts*, a Leica stereomicroscope was used at a standard magnification of 25X. Tumors were only included when large enough to be classified unambiguously.

X<sup>2</sup> 2X2 test was used to evaluate the significant of difference between negative control and other treatments. The relative frequency was calculated as a frequency of induced tumors in treatments with DBP and DOP.

### Neutral red cytotoxicity assay

Neutral red cytotoxicity assay based on the initial protocol described by **Borenfreund and Puerner (1984)** and modified by **Fotakis and Timbrell (2006)** was carried out. Cytotoxicity assay was measured as optical density at 540nm. Dose-response curves were plotted, and 50% inhibitory concentrations of plant extracts (IC<sub>50</sub>) were calculated through Graph Pad Prism software program. For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the LSD test for post hoc analysis. Statistical significance was accepted at a level of  $P < 0.05$ . Data were analyzed using SPSS (version 11; Chicago, IL, USA).

## RESULTS AND DISCUSSION

### Migration compounds profile of bottled water stored under sunlight

Data given in **Table (1)** indicated the migration compounds profile µg/L that detected in water (sample A, PET clear and 0.6L) owing to storage under sunlight for 210 day. Before storage only two compounds (FA and AA) were detected out of nine compounds at 15<sup>th</sup> day of storage all of nine compounds were detected with different concentra-

tions. Both of FA and AA were greatly increased to be 4 and 5µg/L, respectively. Other seven detected compounds that disappeared in zero time were started to be detect, four of them were around 1µg/L (Sb, DEHP, DEP and DBP), meanwhile DOP was 0.4µg/L, and DMP was 0.6µg/L.

During storage under sunlight four compounds (Sb, BPA, DMP and DEP) were increased till 30<sup>th</sup> day then did not affect till the end of storage. Other detected compounds were continuously progressed till the end of storage with different rates. The highest rate was appeared in case of FA it was 222 fold followed by DOP compound (173 fold) as well as DBP (75.3 fold) and finally AA compound with 17.9 fold.

Regarding to another sample B that differed in size (1.5L), **Table (2)** indicated that only 3 compounds (BPA, DMP and DBP) were not detected at zero time. The AA compound was detected with the highest concentration (0.9µg/L) and the lowest one was DOP (0.007µg/L). At 15<sup>th</sup> day of storage BPA and DMP was started to be detect with 0.02 and 0.4 µg/L then increased at 30<sup>th</sup> day and stop increasing till the end of storage. The third compound that detected at 15<sup>th</sup> day (DBP) was 0.7µg/L then sharply progressed to be 74.0µg/L (about 106 fold).

Sb compound was increased till 30<sup>th</sup> day then showed a constant level till the end of storage. Similar trend was recorded in case of DEHP but after 90<sup>th</sup> day of exposure to sunlight. A continuous increase was recorded in other detected compounds (DEP, DOP, FA and AA).

Regarding the effect of bottle's color on migrated compounds concentration during storage under sunlight, **Table (3)** represented such effect in PET blue, 1.5L natural drinking water bottle. Only four compounds were detected before storage named Sb, DOP, FA and AA, the lowest level (0.006µg/L) was noticed in DOP, while the moderate level was recorded in FA (0.03µg/L) and Sb (0.08µg/L). The highest one (0.4µg/L) was recorded in case of AA. It is of interest that two of detected compounds were constant after 30<sup>th</sup> day till the end of storage (210 day), these compounds are Sb and DMP. Other detected compounds; i.e. DBP, DOP, AA and FA were consequently increased with higher levels that were 70.2, 68.8, 37.9 and 24.10µg/L, respectively. On the other hand, three of detected migrated compounds were shown with moderate concentrations ranged between 1.5-5.2µg/L. These compounds are DEP, Sb and DEHP.

**Table 1.** Migrated compounds concentration under sunlight ( $\mu\text{g/L}$ ) for sample A\*

Storage time (day)	Sb	FA	AA	BPA	DEHP	DMP	DEP	DBP	DOP
Zero	0.00 <sup>C</sup>	0.09 <sup>D</sup>	1.82 <sup>D</sup>	0.00 <sup>C</sup>	0.00 <sup>D</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00 <sup>E</sup>	0.00 <sup>E</sup>
15	0.90 <sup>B</sup>	4.00 <sup>C</sup>	5.00 <sup>C</sup>	0.04 <sup>B</sup>	1.20 <sup>C</sup>	0.60 <sup>B</sup>	0.90 <sup>B</sup>	1.00 <sup>D</sup>	0.40 <sup>D</sup>
30	2.60 <sup>A</sup>	19.80 <sup>B</sup>	31.90 <sup>B</sup>	0.05 <sup>A</sup>	4.90 <sup>B</sup>	1.10 <sup>A</sup>	1.20 <sup>A</sup>	2.90 <sup>C</sup>	1.90 <sup>C</sup>
90	2.60 <sup>A</sup>	19.80 <sup>B</sup>	32.0 <sup>B</sup>	0.05 <sup>A</sup>	5.10 <sup>A</sup>	1.10 <sup>A</sup>	1.20 <sup>A</sup>	30.20 <sup>B</sup>	29.10 <sup>B</sup>
150	2.60 <sup>A</sup>	20.0 <sup>A</sup>	32.60 <sup>A</sup>	0.05 <sup>A</sup>	5.10 <sup>A</sup>	1.10 <sup>A</sup>	1.20 <sup>A</sup>	75.30 <sup>A</sup>	69.20 <sup>A</sup>
210	2.60 <sup>A</sup>	20.0 <sup>A</sup>	32.60 <sup>A</sup>	0.05 <sup>A</sup>	5.10 <sup>A</sup>	1.10 <sup>A</sup>	1.20 <sup>A</sup>	75.30 <sup>A</sup>	69.20 <sup>A</sup>

Values followed by the same letter in a column are not significantly different  $P \leq 0.05$

\*See abstract and materials and methods

**Table 2.** Migration compounds concentration under sunlight ( $\mu\text{g/L}$ ) for sample B\*

Storage time/day	Sb	FA	AA	BPA	DEHP	DMP	DEP	DBP	DOP
Zero	0.02 <sup>C</sup>	0.06 <sup>D</sup>	0.90 <sup>C</sup>	0.00 <sup>C</sup>	0.10 <sup>D</sup>	0.00 <sup>C</sup>	0.03 <sup>D</sup>	0.00 <sup>E</sup>	0.007 <sup>E</sup>
15	0.60 <sup>B</sup>	2.00 <sup>C</sup>	4.00 <sup>B</sup>	0.02 <sup>B</sup>	1.10 <sup>C</sup>	0.40 <sup>B</sup>	0.70 <sup>C</sup>	0.70 <sup>E</sup>	0.50 <sup>D</sup>
30	2.10 <sup>A</sup>	17.0 <sup>B</sup>	31.90 <sup>A</sup>	0.04 <sup>A</sup>	4.30 <sup>B</sup>	1.00 <sup>A</sup>	1.10 <sup>B</sup>	2.50 <sup>D</sup>	1.60 <sup>C</sup>
90	2.10 <sup>A</sup>	17.0 <sup>B</sup>	32.0 <sup>A</sup>	0.04 <sup>A</sup>	4.40 <sup>A</sup>	1.00 <sup>A</sup>	1.10 <sup>B</sup>	33.70 <sup>C</sup>	31.60 <sup>B</sup>
150	2.10 <sup>A</sup>	17.30 <sup>B</sup>	32.0 <sup>A</sup>	0.04 <sup>A</sup>	4.40 <sup>A</sup>	1.00 <sup>A</sup>	1.20 <sup>A</sup>	72.00 <sup>B</sup>	66.00 <sup>A</sup>
210	2.10 <sup>A</sup>	18.0 <sup>A</sup>	32.40 <sup>A</sup>	0.04 <sup>A</sup>	4.40 <sup>A</sup>	1.00 <sup>A</sup>	1.20 <sup>A</sup>	74.0 <sup>A</sup>	66.90 <sup>A</sup>

Values followed by the same letter in a column are not significantly different  $P \leq 0.05$

\*See abstract and materials and methods

**Table 3.** Migrated compounds concentration under sunlight ( $\mu\text{g/L}$ ) for sample C\*

Storage time/day	Sb	FA	AA	BPA	DEHP	DMP	DEP	DBP	DOP
Zero	0.08 <sup>D</sup>	0.03 <sup>D</sup>	0.40 <sup>E</sup>	0.0 <sup>C</sup>	0.00 <sup>D</sup>	0.00 <sup>C</sup>	0.00 <sup>D</sup>	0.00 <sup>E</sup>	0.006 <sup>E</sup>
15	1.30 <sup>C</sup>	6.00 <sup>C</sup>	1.00 <sup>D</sup>	0.03 <sup>B</sup>	1.40 <sup>C</sup>	0.90 <sup>B</sup>	1.20 <sup>C</sup>	1.30 <sup>D</sup>	0.80 <sup>D</sup>
30	2.60 <sup>B</sup>	24.0 <sup>B</sup>	37.0 <sup>C</sup>	1.00 <sup>A</sup>	5.00 <sup>B</sup>	1.20 <sup>A</sup>	1.40 <sup>B</sup>	3.60 <sup>C</sup>	2.90 <sup>C</sup>
90	2.60 <sup>B</sup>	24.0 <sup>B</sup>	37.10 <sup>B</sup>	1.00 <sup>A</sup>	5.20 <sup>A</sup>	1.20 <sup>A</sup>	1.40 <sup>B</sup>	33.50 <sup>B</sup>	32.10 <sup>B</sup>
150	2.70 <sup>A</sup>	24.10 <sup>A</sup>	37.90 <sup>A</sup>	1.00 <sup>A</sup>	5.20 <sup>A</sup>	1.20 <sup>A</sup>	1.50 <sup>A</sup>	70.20 <sup>A</sup>	68.80 <sup>A</sup>
210	2.70 <sup>A</sup>	24.10 <sup>A</sup>	37.90 <sup>A</sup>	1.00 <sup>A</sup>	5.20 <sup>A</sup>	1.20 <sup>A</sup>	1.50 <sup>A</sup>	70.20 <sup>A</sup>	68.80 <sup>A</sup>

Values followed by the same letter in a column are not significantly different  $P \leq 0.05$

\*See abstract and materials and methods

When polycarbonate used as a colored bottle contained 19L, **Table (4)** showed absence of five compounds (DMP, DEP, DBP, DOP and FA) before storage. The DEHP and AA compounds came with the same concentration 0.2 $\mu\text{g/L}$  at zero time storage. Similar trend that observed earlier (after

30<sup>th</sup> day) was also recorded in case of DEHP, DMP, DEP and FA. On the other hand each of Sb, BPA, DBP, DOP and AA were continuously increased with different rates till the end of exposure. The higher values were recorded for BPA 52.9 and AA 36.4 $\mu\text{g/L}$ ).

**Table 4.** Migrated compounds concentration under sunlight ( $\mu\text{g/L}$ ) for sample D\*

Storage time/day	Sb	FA	AA	BPA	DEHP	DMP	DEP	DBP	DOP
Zero	0.01 <sup>D</sup>	0.00 <sup>D</sup>	0.20 <sup>E</sup>	0.03 <sup>E</sup>	0.20 <sup>D</sup>	0.00 <sup>C</sup>	0.00 <sup>D</sup>	0.00 <sup>E</sup>	0.00 <sup>E</sup>
15	1.10 <sup>C</sup>	6.00 <sup>C</sup>	0.80 <sup>D</sup>	0.07 <sup>D</sup>	1.40 <sup>C</sup>	0.90 <sup>B</sup>	1.00 <sup>C</sup>	1.20 <sup>D</sup>	0.80 <sup>D</sup>
30	2.40 <sup>B</sup>	21.30 <sup>A</sup>	36.10 <sup>C</sup>	2.60 <sup>C</sup>	4.70 <sup>B</sup>	1.20 <sup>A</sup>	1.30 <sup>B</sup>	3.20 <sup>C</sup>	2.60 <sup>C</sup>
90	2.40 <sup>B</sup>	21.20 <sup>B</sup>	36.30 <sup>B</sup>	22.70 <sup>B</sup>	4.90 <sup>A</sup>	1.20 <sup>A</sup>	1.30 <sup>B</sup>	4.10 <sup>B</sup>	4.80 <sup>B</sup>
150	2.50 <sup>A</sup>	21.30 <sup>A</sup>	36.40 <sup>A</sup>	52.80 <sup>A</sup>	4.90 <sup>A</sup>	1.20 <sup>A</sup>	1.40 <sup>A</sup>	6.30 <sup>A</sup>	5.20 <sup>A</sup>
210	2.50 <sup>A</sup>	21.30 <sup>A</sup>	36.40 <sup>A</sup>	52.90 <sup>A</sup>	4.90 <sup>A</sup>	1.20 <sup>A</sup>	1.40 <sup>A</sup>	6.35 <sup>A</sup>	5.22 <sup>A</sup>

Values followed by the same letter in a column are not significantly different  $P \leq 0.05$

\*See abstract and materials and methods

### Somatic mutation and recombination Tests (SMART) tumor body spots in *Drosophila*

The sensitivity of *wts* tumor suppressor gene for the mutagenic potentialities of Mitomycin C (MMC). In cross, *wts* females (*wts/TM3 Sb*) were crossed to wild type (OR) males. However, F1 larvae (48-56 hour old) were treated with the MMC (20 $\mu\text{g/ml}$  for 24 hour), the data of induced tumors, in addition, to concurrent negative control, are shown in **Table (5)**. The frequencies and distribution of spontaneous and induced tumors after treatment with MMC in both crosses are shown in **Table (5)**. The frequencies of spontaneous tumors in both of negative controls was very low, thirteen small tumors were scored among 310 flies with an average of 0.042 tumor/fly. Meanwhile, After MMC treatment of F1 larvae, the frequency of induced tumors was highly significant increased, whereas, 288 induced tumors were obtained among 230 flies with a frequency of 1.25 tumor/fly. These tumors were found on all body parts and most of them were undifferentiated large tumors. In DOP (30 $\mu\text{g/ml}$ ) and DBP (30 $\mu\text{g/ml}$ ) treatments 158 tumors and 135 were scored, respectively. The overall average of induced tumor was 1.5 and 1.4 tumor/fly, (**Table 5**). However, DOB and DBP treatments were more potent to increase the tumor induction rate in this assay than negative control as illustrated in (**Fig. 1**).

### In vitro assay for cytotoxic activity human cell lines (neutral red assay)

The effects of five different concentrations (0.5, 1, 5, 10 and 20 $\mu\text{g/ml}$ ) of the two phthalate compounds; Di-butyl phthalate (DBP) and di-octyl phthalate (DOP) on the proliferation of liver cancer cells in comparison to a positive control Doxorubicin HCL (3 $\mu\text{g/ml}$ ) were determined using the neu-

tral red cytotoxic assay. In general, the cell viability was decreased gradually as the concentration of the tested phthalates increased (**Table 6**). The cytotoxicity and cell viability of di-octyl phthalate (DOP) and di-butyl phthalate (DBP) with the concentrations (0.5, 1, 5, 10 and 20  $\mu\text{g/ml}$ ) and a positive control 3 $\mu\text{g/ml}$  were evaluated in vitro against human liver cell lines (hepatoma cells HepG2). The viability of positive control was 62.85%, and the viability of HepG2 was reduced as the concentration increased of the tested phthalates, but the reduction was non-significant in 0.5 $\mu\text{g/ml}$ . The significant reduction in the viability was observed in 1 and 5 $\mu\text{g/ml}$ , moreover, highly significant in 10 and 20 $\mu\text{g/ml}$ . The Dose inducing 50% cell growth inhibition (IC50) against hepatoma cell line cells (HepG2) are presented in **Table (6)**.

Results indicated that the use of phthalates may cause potential health risks to human beings **Chen et al (2014)**.

**Boekelheide et al (2009)** concluded that DBP inhibited proliferation, because they exposed somatic cells in the fetal rat testis with DBP, and the results decreased proliferation, rather than increased apoptosis. DBP may cause genetic defects in male gametes, which may deteriorate sperm quality of male offspring, and delayed sexual maturation of female offspring (**Dobrzyńska et al 2011**).

**Woźtowicz et al (2017)** demonstrated that DBP- increased ROS production at a concentration of 10nm, while it activated LDH activities at 1 $\mu\text{M}$  and apoptosis (programmed cell death). Moreover, cytotoxic effect (decreased cell viability) was observed. And found the toxicity in rat embryonic midbrain and mesencephalic neurospheres. **Abdel-Ghani et al (2014)** observed the decreases in fertility of male and female rats, will increase mortality rate with DNA damage. DBP has hepatotoxicity effect, increased of GOT and GPT activity

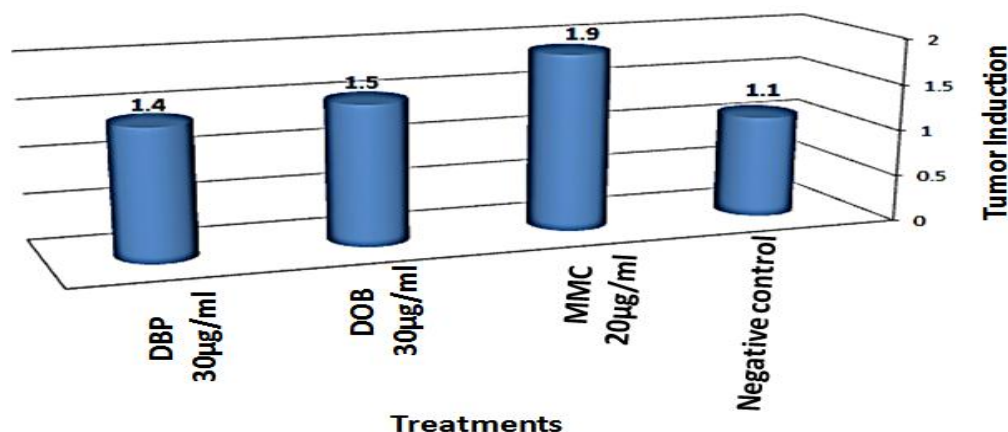
**Table 5.** Frequencies of induced tumor in trans-heterozygous (*wts/+*) after larvae feeding treatments with concentrations of DOP (di-octyl phthalate) and DBP (Di-butyl phthalate) comparing with the MMC as a positive control and negative control

Treatments	Total No. of fly scored	No. of fly scored with tumor	No. of tumor scored	Tumor induction	Frequency (No. of Tumor/fly)
Negative control	310	12	13	1.1	0.042
MMC 20µg/ml	230	151	288	1.9	1.25
DOB 30µg/ml	250	102	158	1.5	0.63*
DBP 30µg/ml	271	99	135	1.4	0.5*

\* significant difference from the negative control at  $P < 0.05$  using Mann, Whitney and Wilcoxon nonparametric *U* test.

Frequency (No. of Tumor/fly) = Number of tumors/Total number of tested flies.

Tumor induction = Number of tumors/ Number of tumor flies.



**Fig. 1.** Diagram represents the tumor induction of spontaneous and induced warts epithelial tumors in *+wts* flies after treatments with mitomycin C (MMC), DOB and DBP.

**Table 6.** The cell viability percentage and IC50 of liver cancer human cell lines tested by DOB and DBP compared with Positive control using neutral red cytotoxicity assay

Human cell line	Concentration (µg/ml)	Viability %		Positive control (3 µg/ml)
		DOB	DBP	
Liver cancer (Hep G2)	0.5 µl/ml	85.46	92.7	62.85
	1 µl/ml	55.9*	65.5*	
	5 µl/ml	51.98*	46.6**	
	10 µl/ml	31.9**	39.5**	
	20 µl/ml	13.8**	21.4**	
IC50		4.376	3.076	

\* and \*\* significant and highly significant difference from the negative control at  $P < 0.05$  using one-way analysis of variance (ANOVA).

was measurement. Moreover, embryonic, developmental, reproductive toxicity (Chen et al 2014) and potential carcinogenicity was reported. The results agree with Kim et al (2002), they suggest that DBP and DOP induced developmental toxicity in rat embryonic limb bud cells. The IC50 values of DBP for cytotoxicity in neutral red assay was 25.54µg/ml and cell differentiation was 21.21µg/ml. The treatment rats with DOP at two to four weeks increased liver replicative DNA synthesis and liver function enzymes were observed (Smith et al 2000).

### CONCLUSIONS

The effect of sunlight exposure on chemicals release into PET- bottled water and the potential hazard of water extracts were investigated using in vitro assay for cytotoxic activity human cell lines. The migration of aldehydes, phthalates and Sb into water increased with exposure of sunlight especially after 30<sup>th</sup> day of exposure. Generally, hazardous and undesirable risks could happen due to prolonged photo-degradation and leaching of some carcinogenic chemical compounds from the plastic materials of the bottles. Therefore, this study recommended that PET- bottled water should be only once used and stored in a cool, dry places in the absence of sunlight to avoid health hazards from chemical contamination..

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## التأثير الوراثي للمركبات المهاجرة من مياه الشرب الطبيعية المعبأة والمخزنة تحت أشعة الشمس المباشرة

[140]

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البولى كربونات وسعتها 19 لتر) لدراسة تأثير التعرض لأشعة الشمس المباشرة على هجرة كل من عنصر الأنتيمون وكذلك مجموعة مركبات الفيتالات. وأجريت التجربة بالتعريض لأشعة الشمس (7 ساعات يومياً) لمدة 210 يوماً. وأظهر الشكل العام للمركبات المهاجرة (ميكروجرام/لتر) للعينة (A) قبل التخزين وجود مركبين فقط (الفورمالدهيد والأسيتالدهيد) من تسع مركبات وبالتخزين تحت أشعة الشمس إزداد إلى أربع مركبات (الانتيمون، البيسفينول أ، الداى ميثيل فيتالات، الداى إيثيل فيتالات). حتى اليوم الثلاثون ثم لم يتأثر وجودهم بعد ذلك حتى نهاية فترة التخزين. واستمرت الزيادة فى المركبات الأخرى التى قدرت حتى نهاية فترة التخزين ولكن بمعدلات مختلفة وظهر أعلى معدل فى حالة الفورمالدهيد حيث كان بزيادة مقدارها (222 ضعفاً) ثم مركب الداى أوكثيل فيتالات (173 ضعفاً) ومركب الداى بيوتيل فيتالات (75.3 ضعفاً) وأخيراً مركب الأسيتالدهيد بمقدار (17.9 ضعفاً). وبالنسبة للعينة الثانية (B) لم يتم إكتشاف ثلاثة مركبات (داى بيوتيل فيتالات، داى ميثيل فيتالات، البيسفينول أ) قبل التخزين. وتم ظهور مركب الأسيتالدهيد كأعلى تركيز (0.9 ميكروجرام/لتر) بينما ظهر مركب الداى أوكثيل فيتالات كأقل تركيز (0.007 ميكروجرام/لتر) وظهر قبل التخزين أربع مركبات فقط هما (الانتيمون، الداى

الكلمات الدالة: عبوات البولى إيثيلين تيريفيتالات، المياه المعبأة، أشعة الشمس، الفيتالات، الهجرة، السمية، مواد مسرطنة، تأثيرات السمية الوراثية

### الموجز

تعتبر مياه الشرب الطبيعية واحدة من أهم مسببات تعرض الإنسان للملوثات من خلال تحرر عنصر الأنتيمون وكذا البوليمرات من العبوة البلاستيكية المصنعة من البولى إيثيلين تيريفيتالات (PET). ويهدف هذا البحث إلى دراسة تأثير أشعة الشمس على هجرة المركبات الكيميائية إلى مياه الشرب المعبأة فى عبوات الـ (PET) وكذلك دراسة السمية الخلوية بمركبى الداى بيوتيل فيتالات (DBP) والداى أوكثيل فيتالات (DOP) بواسطة إختبار قياس الطفرات الجسمية والعبور الوراثي (SMART) فى حشرة الدروسوفيل. وتمت الدراسة على أربع عينات عشوائية من مياه الشرب المعبأة والمنتجة بواسطة شركات تصنيع مختلفة وتم تكويد العينات كالتالى:

العينة (A) مصنعة من الـ PET الشفاف وسعتها 0.6 لتر، العينة (B) مصنعة من الـ PET الشفاف وسعتها 1.5 لتر، العينة (C) مصنعة من الـ PET الأزرق وسعتها 1.5 لتر، العينة (D) مصنعة من

المسرطنة؛ مثل خلايا سرطان الكبد (HepG2)، خلايا سرطان القولون (HCT-116) وتقييم السمية الخلوية بإختبار السمية الخلوية الحمراء المعتدلة. علاوة على ذلك، تم تقييم القدرة على إحداث التسرطن بإستخدام إختبار قياس الطفرات الجسمية والعبور الوراثي (SMART) في الدروسوفيلا. وأظهرت النتائج تحفيز تكوين الأورام وزيادة معدلها مقارنة بالعينة المرجعية (الكنترول)، كما حدث خفض في حيوية خلايا كبد الإنسان المستخدمة وذلك عند تركيزات 0.5، 1.0، 5، 10 و 20 ميكروجرام/مل ولم يظهر أي تأثير على خلايا القولون.

أوكنتيل فيثالات، الفورمالدهيد، الأسيتالدهيد). وكان أقل تركيز هو لمركب الداى أوكنتيل فيثالات (0.006 ميكروجرام/لتر) بينما سجل مركب الفورمالدهيد بقيمة متوسطة (0.03 ميكروجرام/لتر). وكذلك مركب الأنتيمون (0.08 ميكروجرام/لتر). أما المركبات الأخرى التى قدرت وهى (الداى بيوتيل فيثالات، الداى أوكنتيل فيثالات، الفورمالدهيد، الأسيتالدهيد) فقد إزدادت بإستمرار بقيم أعلى.

وفى هذه الدراسة تم أيضاً تقييم مركبى الداى بيوتيل فيثالات والداى أوكنتيل فيثالات بتركيز 30 ميكروجرام/مل على بعض خطوط خلايا الإنسان



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