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# Impact of Five Organic UV Filters on the Multixenobiotic Resistance Mechanism of Human Embryonic Lung Fibroblasts

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**Abstract.** Organic UV filters continuously accumulate after entering the environment, thus posing potential ecological and health risks. The impact of five organic UV filters, namely BP-3, BP-4, OC, EHMC, and BM-DBM, on the multixenobiotic resistance (MXR) mechanism in human embryonic lung fibroblasts (MRC-5) was investigated to identify the toxic effects of inhaled UV filters on human health. Results showed that OC exerted a more significant inhibitory effect on the transport proteins of MRC-5 cells than the chemical sensitizer VER. Moreover, inhibition times significantly increased with the increasing exposure concentration of UV filters. BM-DBM exerted a significant inhibitory effect at exposure concentrations of 10 and 100μmol/L. The results of this study provide powerful scientific basis for the investigation of the impact of UV filters on the MXR mechanism of human cells at the molecular level.

## 1. Introduction

In recent years, UV filters have been widely added to various personal care products (PCPs) and have become a new class of emerging pollutants given their constant entry into the environment. Organic UV filters account for the vast majority of UV filters that contaminate the environment. Certain organic UV filters act as endocrine disruptors and exert developmental and genotoxic effects, thus posing a potential risk to ecosystem and human health [1–3]. Existing reports have mainly focused on the toxic effects of UV filters on daphnia, fish, other aquatic organisms, and rats [4–6]. Moreover, several reports have focused on the impact of these compounds on human or mouse cancer cells, germ cells, or embryonic stem cells [7, 8]. Humans, particularly children, are exposed to organic UV filters through the inhalation of contaminated atmospheric and indoor dust. The effects of inhaled UV filters on human health, however, are rarely reported. The toxic effect of low doses of organic UV filters on non-target organs, such as the lung, is unclear and needs further exploration.

Organisms can form a barrier at the tissue–environment interface to prevent exogenous compounds from entering cells through the action of biological pump transporters. Biological pump transporters can identify and expel exogenous compounds that have erroneously entered

cells [9]. Toxins can accumulate in cells and exert toxic effects when the activity of biological pump transporters is inhibited [10–12]. The protective mechanism of biological pump transporters is known as multixenobiotic resistance (MXR) and has attracted widespread attention in recent ecotoxicological studies. However, the impact of organic UV filters on the MXR mechanism has yet to be reported.

This study aims to provide a scientific basis for understanding and evaluating the potential risks of organic UV filters to human health and to represent their effects on the MRX mechanism at the molecular level. The effects of five types of common organic UV filters, namely, BP-3, BP-4, OC, EHMC, and BM-DBM, on the human embryonic lung fibroblast cell line MRC-5 are investigated in this study. Rhodamine-123 (Rh-123) is selected as a fluorescent substrate for biological pump transporters, and a chemical sensitizer VER is used as a positive control.

## 2. Materials and Method

### 2.1 Experiment Materials

The MRC-5 cell line was purchased from the ATCC Cell Bank. The culture medium was prepared by mixing 500mL Minimum Essential Medium (MEM), (+L-glutamine, +Phenol Red, and -HEPES; Gibco, USA), 5mL double antibody–penicillin–streptomycin (+10,000 Units•$mL^{-1}$ Penicillin, +10 000μg•$mL^{-1}$ streptomycin, Gibco, USA), and 50 mL PBS buffer (-Calcium, -Magnesium, and -Phenol Red; Gibco, USA).

The following reagents were used: BP-3 (98%, Sigma-Aldrich, USA), BP-4 (98%, Sigma–Aldrich, USA), OC (97%, Sigma-Aldrich, USA), EHMC (96%, TCI, Japan), BM-DBM (98%, TCI, Japan), dimethyl sulfoxide (DMSO, 99%, Shanghai Sangon), Rh-123 (Sigma-Aldrich, USA), and VER (Sigma–Aldrich, USA).

The following instruments were used: Thermo Varioskan Flash full-wavelength multifunctional enzyme spectrometer (Thermo, USA), 3K30-type high-speed centrifuge (Sigma, USA), YXQ-LS-18S type automatic portable sterilizing pot (Shanghai Boxun), and DSH-series Super-clean bench program-controlled instrument (Shanghai Dinshanhu).

### 2.2 Experimental Method

To prepare stock solutions, the five organic UV filters were separately dissolved in DMSO to a final concentration of 100mmol/L. Before the experiment, the solutions were diluted to 1, 10, and 100μmol/L for use as working exposure fluids.

MRC-5 cells that were frozen in liquid nitrogen were resuscitated and re-suspended in ultrapure water at 37ºC. The cell suspension was then transferred to a centrifuge tube. Then, the cryopreserved cells were diluted with 5mL medium and collected through centrifugation (1000 rpm, 5 min). The supernatant was then removed. The cells were then suspended in 1mL medium and evenly dispersed through repeated trituration with a pipette. Approximately 300–400 μL of the cell suspension was obtained and inoculated in 6mL medium. Cell culture flasks were cultured in a $CO_2$ incubator (5% (v/v) $CO_2$/air, 37ºC).

The exposure test was conducted with four groups of cells: the experimental group (exposure to working fluid), negative control group (exposure to 1% whole DMSO medium), positive control group (exposure to VER), and blank control group (0.01mol/L PBS). MRC-5 cells in the logarithmic phase were first digested with trypsin. Then, the prepared MEM medium was

pipetted to yield a single-cell suspension. The supernatant was aspirated and removed after centrifugation (1000r/min, 5min). After the addition of PBS, the suspension was triturated using a pipette to obtain a cell suspension with a density of $1\times10^6$ cells/mL. The cell suspension was inoculated in 1.5mL Eppendorf pipes with 1mL of suspension per tube. The supernatant was removed after centrifugation (1000 r/min, 5 min). Then, the exposure working liquid or MEM culture medium was added to the treatment groups, and the suspensions were placed in an incubator for 1 h and then on ice to terminate growth. The suspensions were centrifuged at 4ºC (2500 r/min, 5 min), and the supernatants were removed. The cells were washed twice with cold PBS. Rh-123 solution was added to the experimental and control groups. Then, 1mL ultrapure water was added to each group, and the suspensions were placed in an incubator for 1h. After removal, the suspensions were placed on ice to terminate transfer. The suspensions were centrifuged at 4ºC (2500r/min, 5min). The supernatant was removed, the precipitate was washed twice with PBS, and then centrifuged again at 4 ℃. Then, 1mL PBS was added to the precipitate was added after the supernatant was removed. The cell suspension was then triturated again and then transferred to a 96-well plate. A multifunctional microplate reader was used to detect fluorescence intensity in cells. The excitation and emission wavelengths were 535 and 466 nm, respectively.

Before the actual experiment, numerous experiments were conducted to observe whether the UV filters interfered with the fluorescence emission of Rh-123. All experimental solutions were added into test tubes and mixed in a vortex electron-shock instrument. Then, solutions were added to a 96-well plate at 200μL per well. The blank control group was set. The multifunctional microplate reader was used to detect the absorbance optical density (OD) of all groups with excitation and emission wavelengths of 466 and 535 nm, respectively.

### 2.3 Data and Statistical Analysis

Experimental data were recorded as mean value + standard deviation (SD). Data were analyzed with SPSS software (IBM SPSS Statistics 20.0). Single-factor analysis of variance (ANOVA) and multiple range test (LSD) were used to analyze the difference between the control and experimental groups. $p < 0.05$ indicated significant difference.

## 3. Results

### 3.1 Fluorescence Quenching Result

The maximum concentration of the subjects in each group was selected. The *t*-test was conducted to observe the difference between the absorbance of each group and that of the Rh-123 control group. If $p > 0.05$, no significant difference in fluorescence intensity was observed between the experimental and control groups, as shown in Tab. 1. This result indicated that the subjects in this study were within the target concentration range and that organic UV filters do not exert a fluorescence enhancing or quenching effect.

Table 1. Quenching effects of UV filters and VER.

| Drugs | fluorescence intensity ($n$ =5) | | | | $p^b$ |
|---|---|---|---|---|---|
| | Experimental group | Control group | Difference | Rh-123 control group | |
| BP-3 | 886.89 | 0.20 | 886.68 | 701.56 | >0.05 |
| BP-4 | 518.19 | 0.05 | 518.14 | 701.56 | >0.05 |
| OC | 857.88 | 0.11 | 857.78 | 701.56 | >0.05 |
| EHMC | 747.30 | 0.04 | 747.26 | 701.56 | >0.05 |
| BM-DBM | 1003.54 | 3.54 | 1000.00 | 701.56 | >0.05 |
| VER | 805.88 | 0.04 | 805.84 | 701.56 | >0.05 |

a Difference in fluorescence intensity between subjects in the experimental and control groups. b *t*-test conducted to determine the difference in fluorescence between the experimental and Rh-123 control groups.

### 3.2 Impact of Five Types of UV Filters on the MXR Mechanism of MRC-5 Cells

The comparative test result showed that the fluorescence intensity of VER positive control group increased by nearly 1-fold that of the negative control group. This result indicated that VER exerts a significant inhibitory effect on the activity of transport proteins and restricts the efflux of Rh-123 from cells.

Table 2. Effects of 5 UV filters on the efflux of Rh-123 from MRC-5 cells.

| Group | Concentration (μmol/L) | OD | Increase in OD (%) |
|---|---|---|---|
| Negative control | 0 | 18.09±0.11 | -- |
| VER positive control | 5 | 36.10±0.38[a] | 99.59[a] |

[a] $p < 0.05$

The fluorescence intensity of Rh-123 substrates under the set exposure concentration of the five UV filters is shown as Fig. 1. The fluorescence intensity of the substrates under three exposure concentrations of OC significantly increased; that under two exposure concentrations of BP-3, EHMC, and BM-DBM was significantly enhanced; and that under one exposure concentration of BP4 significantly increased. These results indicated that at the set exposure concentrations, organic UV filters could restrict the efflux of Rh-123 from the cells and inhibit the activity of transport proteins.

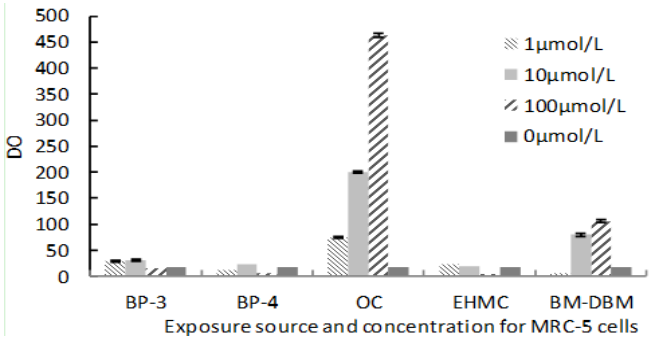


Figure 1. Inhibitory effect of five types of UV filters on the efflux of Rh-123 from MRC-5 cells.

The inhibitory effect of VER on the efflux of Rh-123 was defined as one standard unit to intuitively represent the effect of the five organic UV filters on the MXR mechanism of MRC-5 cells. The inhibition time *X* of all exposure groups were calculated on the basis of the *X* of the VER positive group, as shown in Fig. 2. OC exerted the strongest inhibitory effect on the efflux of Rh-123 from cells. *X* reached 2 under 1μmol/L OC. Then, with the increase in exposure concentration, *X* continuously increased to 13 under 100μmol/L OC. The inhibitory effect of BM-DBM on the efflux of Rh-123 from cells increased with increasing exposure concentration. Under 10μmol/L BM-DBM, *X* exceeded 2. The inhibitory effects of BP-3 and BP-4 initially increased and then gradually decreased with increasing exposure concentration. Under all exposure concentrations, *X* was less than 1. The inhibitory effect of EHMC decreased with increasing exposure concentration. Moreover, *X* was less than 1 under all exposure concentrations.

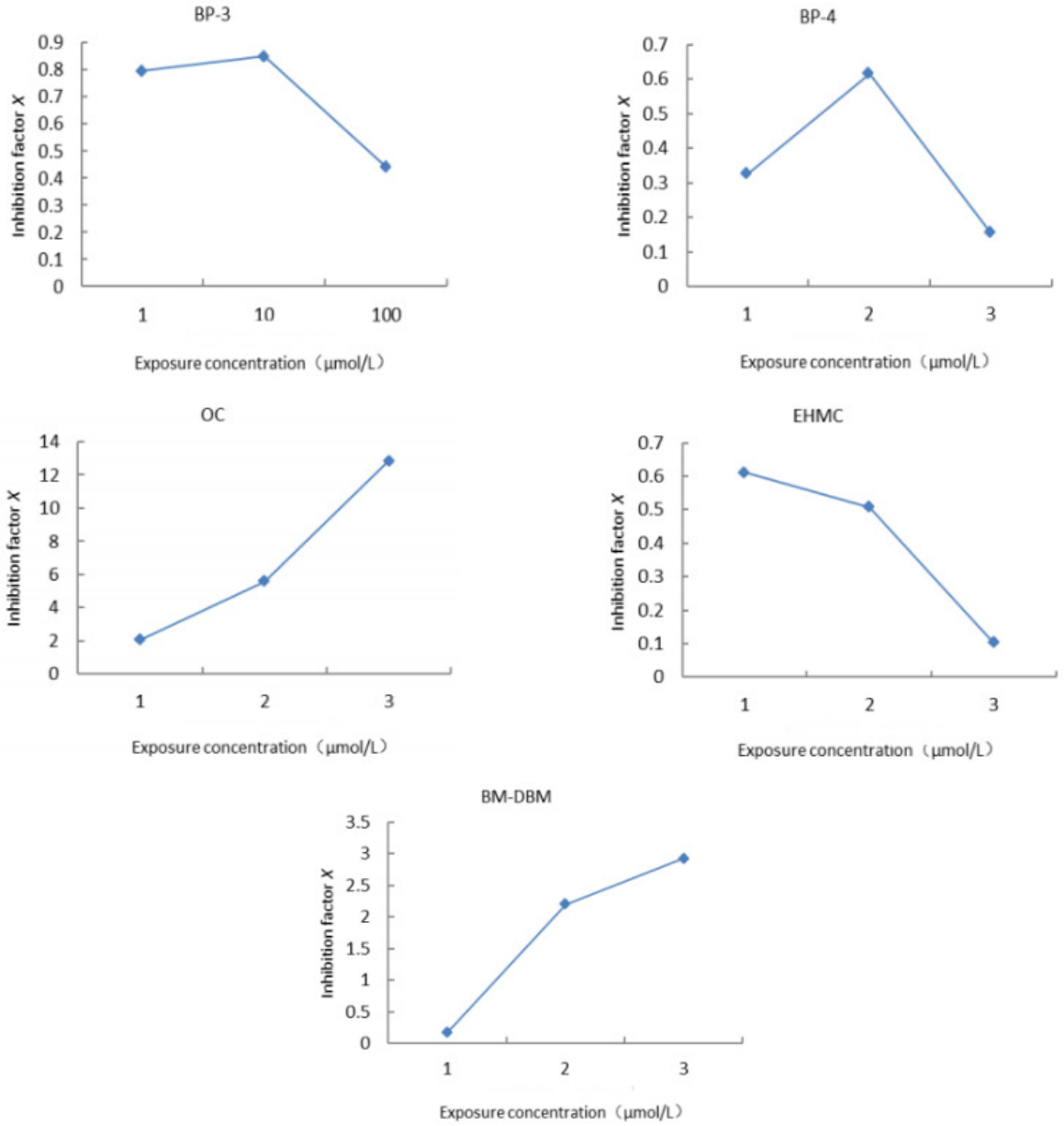


Figure 2. Inhibition time *X* under different exposure concentrations of the five organic UV filters.

## 4. Discussion

Organic UV filters are present in various environmental media. The residual concentration of UV filters in the environment continually increases given their recalcitrance. Owing to their potential health risk, these compounds should be further studied. At present, most related studies on organic UV filters have focused on the water environment, and the subjects mainly include fish, invertebrates, and algae. Moreover, studies on the toxicity of organic UV filters have mainly focused on their endocrine-disrupting effects. No related study on the impact of inhalation exposure of organic UV filters on human health has been published.

Substrates accumulate in mammalian cells when chemical sensitizers inhibit the activity of biological pump transport proteins. VER is a common type of sensitizer with an exclusive inhibitory effect on the P-gp protein. VER was regarded as a positive control group in this study. The results of this study showed that VER inhibits the efflux of Rh-123 from MRC-5 cells, leading to enrichment of Rh-123 in cells.

The inhibitory effects of five organic UV filters on the activity of MRC-5 cell transport protein were compared with that of VER. OC, a chemical sensitizer, exerts a more significant inhibitory effect on MRC-5 cells than VER. The inhibitory effect of OC is positively correlated with exposure concentration. At an exposure concentration of 1μmol/L, OC enriched cellular Rh-123 by four times and by 26 times at the exposure concentration of 100μmol/L. OC is the organic UV filter with the highest concentration in environmental media. Negreira et al. [13] analyzed six UV filters present in dust and found that OC is present with the highest concentration of 41μg/g. The maximum detectable concentration of OC in rivers, lakes, and sediments is 4256, 4381, and 2400ng/L, respectively. Moreover, OC is added to PCPs at the additive content of 10% or higher. This study shows that OC exhibits strong chemical sensitization. Combined with its extensive accumulation in environmental media, OC presents the highest potential risk to human health.

Compared with 1μmol/L VER, 1μmol/L BM-DBM exerts weaker inhibitory effect on MRC-5 cells. However, its inhibitory effect is strengthened with increasing concentration. At the concentration of 100μmol/L, its inhibitory effect is thrice that of VER. Rodil et al. found that the concentration of BM-DBM in lakes is 2431ng/L, indicating that organic UV filters accumulate in environmental media. Therefore, the potential risk of BM-DBM to human health increases with increasing concentration.

The inhibitory effects BP-3, BP-4, and EHMC on the efflux of Rh-123 from MRC-5 cells do not show an increasing trend with the increasing exposure concentration. Moreover, under a set exposure concentration, the chemical sensitization of the three UV filters is lower than that of VER. The concentration of EHMC is negatively correlated with the efflux of Rh-123 from MRC-5 cells.

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