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Effects of phthalates on marine organisms: cytotoxicity and genotoxicity of mono-(2-ethylhexyl)-phthalate (MEHP) on European sea bass (*Dicentrarchus labrax*) embryonic cell line

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Abstract

Introduction. Mono-(2-ethylhexyl) phthalate (MEHP) represents a toxicological risk for marine organisms due to its widespread presence in aquatic environments.

Methods. MEHP effects on cell viability, cell death and genotoxicity were investigated on the DLEC cell line, derived from early embryos of the European sea bass *Dicentrarchus labrax* L.

Results. A dose-dependent cytotoxic effect, with no induction of necrotic process, except at its highest concentration, was observed. Moreover, chromosomal instability was detected, both in binucleated and mononucleated cells, coupled with a minor inhibition of cell proliferation, whereas genomic instability was not revealed. To our knowledge, the overall results suggest the first evidence of a possible aneugenic effect of this compound in the DLEC cell line, that is the induction of chromosomal loss events without the induction of primary DNA damage.

Conclusions. MEHP should be considered more harmful than its parent compound DEHP, because it induces genomic instability in the DLEC cell line without triggering cell death.

Key words

- MEHP
- European sea bass cell line
- cytotoxicity
- genotoxicity
- micronuclei

INTRODUCTION

Phthalates (PAEs) are generally used in plastics, fertilizer, pesticides, toys, cosmetics and other industries and they can promote the plasticity, durability and strength of materials [1, 2]. Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzylbuthl phthalate (BzBP), dicyclohexylphthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), dinhexyl phthalate (DnHP), and di-n-octyl phthalate (DnOP) are the most commonly used PAEs in consumer products [3]. In aquatic environments, PAEs can be readily degraded by hydrolysis, photodegradation and microbial degradation [4, 5], or they can come into direct contact with aquatic organisms, entering the food chain and being transported through the trophic levels, ultimately becoming a threat to humans as consumers of aquatic resources [5-7]. When PAEs are ingested by organisms, they are easily metabolized [5]. Most PAEs metabolites are fat-soluble and can be stored in biological tissues for long times [3, 5], up to 6 months, eventually becoming toxic to the organisms [5, 8].

Among PAEs metabolites, mono-(2-ethylhexyl) phthalate (MEHP) is one of the most studied due to its widespread presence in aquatic environments and its toxicological risk [5]. Indeed, several studies have reported that the average concentration of MEHP in superficial neustonic/planktonic samples of the Tyrrhenian Sea ranged from 29.17 ng/g to 93.37 ng/g [9], whereas its concentration in samples collected from the Sea of Cortez (La Paz Bay) ranged from 13.08 ng/g to 13.69 ng/g [9]. Recent studies have also shown that fresh algae and cyanobacteria produce and re-

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lease MEHP under natural conditions, metabolizing the parent compound, DEHP, uptaken directly from the aquatic environment [6]. Nevertheless, European regulations concern the parent compound DEHP and not its primary metabolite MEHP [10]. Thus, to date, a limit for exposure to MEHP in the aquatic environment has not yet been identified.

Similarly to most phthalates, MEHP can adversely affect the developmental and reproductive functions of several organisms, alter the number of offspring produced, reduce hatching success and disrupt larval development [5, 11-13]. Among its major effects, MEHP is known to cause the impairment of reproductive success [14], in particular interfering with androgenic activity [12, 15] and the expression of both sex hormone receptors [12, 14] and steroidogenesis-related genes [12, 15, 16]. Furthermore, MEHP is known to induce apoptosis [13], and have genotoxic, mutagenic, and carcinogenic effects [5, 17] on human and rodent cell lines. However, to our knowledge, very little data is available on the toxic and genotoxic effects of MEHP on aquatic organisms, especially on marine fishes [12].

The European sea bass (*Dicentrarchus labrax*, L. 1758) is an euryhaline marine teleost, which primarily inhabits estuaries, lagoons and coastal waters. This species is of high commercial and recreational value and is one of the most cultivated by the aquaculture industry in the Mediterranean basin [18]. In addition, *D. labrax* plays an important role in trophic networks, and in particular those of a large number of European estuaries and coastal areas [19]. As are most carnivorous species, *D. labrax* is highly exposed to the ingestion of anthropogenic pollutants [6]. Thus, the European sea bass is considered a good bioindicator of marine pollution [19]. In recent decades, fish cell lines have shown to be a reliable tool to assess the cytotoxicity, genotoxicity, gene regulation, virology and tumorigenesis of many pollutants [19, 20]. To date, the European sea bass embryonic cell line (DLEC) [20, 21], formed by fibroblast-like adherent cells, has been shown to be a useful instrument for *in vitro* assessment of toxic compounds [20].

In our previous study [20], we demonstrated that DEHP has a toxic effect on the DLEC cell line, resulting in a significant decrease in cell viability, a moderate increase in DNA strand break, and a dose-dependent increase in the frequency of micronuclei (MN) coupled with a significant and progressive decrease in cell proliferation. Considering that different studies [5, 12, 13] have shown the high toxicity of MEHP, the objective of this study was to evaluate the potential adverse effects of increasing concentrations of MEHP on the DLEC cell line, using specific *in vitro* tests to evaluate MEHP cytotoxicity, genotoxicity, and potential mutagenicity. The DLEC cell line was chosen because of the economical and environmental value of *D. labrax*, and also because of its easy maintenance in laboratory conditions and reliability for *in vitro* applications [21].

MEHP is the primary biologically active metabolite of DEHP, the latter having a short half-life. Moreover, since MEHP remains in the aquatic ecosystem for long periods [22], its occurrence and effects in the aquatic environment have been targeted as biomarkers [12] as well as a tracer of the intake of microplastics due to its high concentration in the blubber of stranded fin whales [9]. Besides, quite high MEHP concentrations have also been found in human samples [23] such as maternal serum (42.6 µM) and umbilical cord serum (37.5 µM). Finally, the accumulative exposure could be even higher considering the continuous contact of the organisms with this phthalate. In this context, MEHP effects should be more thoroughly investigated since exposure to MEHP poses a risk not only to aquatic organisms but to humans as well.

MATERIALS AND METHODS

Chemicals

Leibovitz (L-15) medium without L-glutamine, phosphate buffer saline (PBS) without calcium and magnesium and L-glutamine were purchased from Lonza, Italy. Penicillin/streptomycin, trypsin-EDTA in PBS without calcium, magnesium and phenol red were acquired from EuroClone, Italy. MEHP, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cytochalasin B (1200 µg/mL), trypan blue solution (0.4%), dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (SDS) were bought from Sigma-Aldrich, Italy, while foetal bovine serum (FBS) was purchased from Invitrogen, Italy. MEHP was dissolved in DMSO to obtain a stock solution of 100 mM

Cell culture and MEHP treatments

The European sea bass (*Dicentrarchus labrax* L.) embryonic (DLEC) cell line [21] was used to assess MEHP cytotoxicity and genotoxicity. DLEC cells were maintained at 20-22 °C, without CO_2, in L-15 medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin.

For the positive control 100 µM H_2O_2 was used, while the solvent sample was treated with 1% DMSO for 24 h. Cells were treated for 24 h with different MEHP concentrations ranging from 0.5 to 100 µM. The working concentrations were freshly prepared in DMSO before treatments from MEHP stock solutions. DMSO never exceeded 1% v/v for both treatments and solvent control. For both cytotoxicity and genotoxicity assays, two independent experiments were performed.

Cell viability assay and cell death

DLEC cells were treated with MEHP for 24 h at concentrations of 1, 5, 10, 50 and 100 µM to assess both cell viability and cell death. Cytotoxicity of MEHP was evaluated as per the standard protocols by MTT and Trypan Blue Exclusion (TBE) assays [20, 24]. Briefly, for the former, MTT solution (0.5 mg/ml per well) was added at the end of MEHP treatment and, after additional 3 h of incubation at 21 °C, cells were lysed (10% SDS, 0.6% acetic acid in DMSO) to dissolve the formazan crystals. The spectrophotometer DTX 880 Multimode Detector (Beckman Coulter) was used to measure optical density. Instead, for the TBE assay, cells were harvested after MEHP treatment and cell suspensions were mixed with Trypan Blue solution (1/1, v/v) for 5 minutes, seeded on a slide and evaluated under an optical microscope.

Cell death was measured by fluorescence microscopy, evaluating the pattern of chromatin fragmentation [20, 25, 26]. At the end of MEHP treatment, cells were harvested and cell suspensions were stained with a combination of Fluorescein Di-Acetate (FDA, 0.75 mg/mL), Propidium Iodide (PI, 0.25 mg/mL), and Hoechst (HO, 0.1 mg/mL) dyes before cell death analysis.

Single cell gel electrophoresis

The DLEC cell line was treated with 1, 5, 10, 50 and 100 µM of MEHP for 24 h and the standard alkaline (pH>13) SCGE was performed according to previous works [20, 27]. After slide preparation and cell lysis (2.5 M NaCl, 10 mM Tris-HCl, 100 mM EDTA, pH 10, with 1% Triton X-100 and 10% DMSO freshly added), electrophoresis was conducted for 20 minutes at 25 V and 300 mA at 4 °C preceded by a 15-minute incubation in electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13) to allow DNA unwinding. Slides were then neutralized (0.4 M Tris-HCl, pH 7.5), and stained with ethidium bromide (20 µg/mL, 50 µL). Nucleoids were examined at 400× magnification with a fluorescence microscope (Axioskop 2, Zeiss) associated with a Comet assay III program. For each experimental point, three operators scored a total of 300 randomly-selected cells. Computer-generated % DNA in the tail (tail intensity, TI) values were used to evaluate the amount of primary DNA damage.

Cytokinesis-block micronucleus assay

The DLEC cell line was treated with 0.5, 1, 5, and 10 µM of MEHP for 24 h. The cytokinesis-block micronucleus (CBMN) assay was carried out with the standard technique proposed by Fenech [28]. Cytochalasin B was added after MEHP treatment, lowering the concentration to 2 µg/mL and arresting cell cytokinesis for 48 h. Harvesting and fixing were carried out as previously described [20, 29]. Slides were stained for 10 min with 5% Giemsa. Micronuclei (MN) were scored in both 1000-mononucleated and 1000-binucleated cells with intact cytoplasm for each experimental point. Cell cycle progression analysis was assessed calculating the cytokinesis block proliferation index (CBPI) as previously described [20, 30]. Subsequently, the percentage of cytostasis was calculated according to Lorge and coworkers [30].

Statistical analysis

The comparison between MEHP treatments and solvent was performed by carrying out one-way ANOVA, followed by Sidak's or Tukey's multiple comparisons post-test, for viability tests (MTT, TBE) and apoptotic/necrotic cell death assay; by means of χ^2-test for the cytostatic effect (CBMN); and using Student's *t*-test for paired samples for mean TI (Comet assay) and yield of micronuclei per cell (CBMN). The statistical significance for H_2O_2 and solvent samples was evaluated by comparison to untreated control (medium) as mentioned above. The level for statistical significance was set at $p<0.05$.

RESULTS

Cell viability and cell death

Results of cell viability measured by MTT and TBE assays after 24 h of MEHP treatment are illustrated in *Figure 1*. After treatment with different MEHP concen-

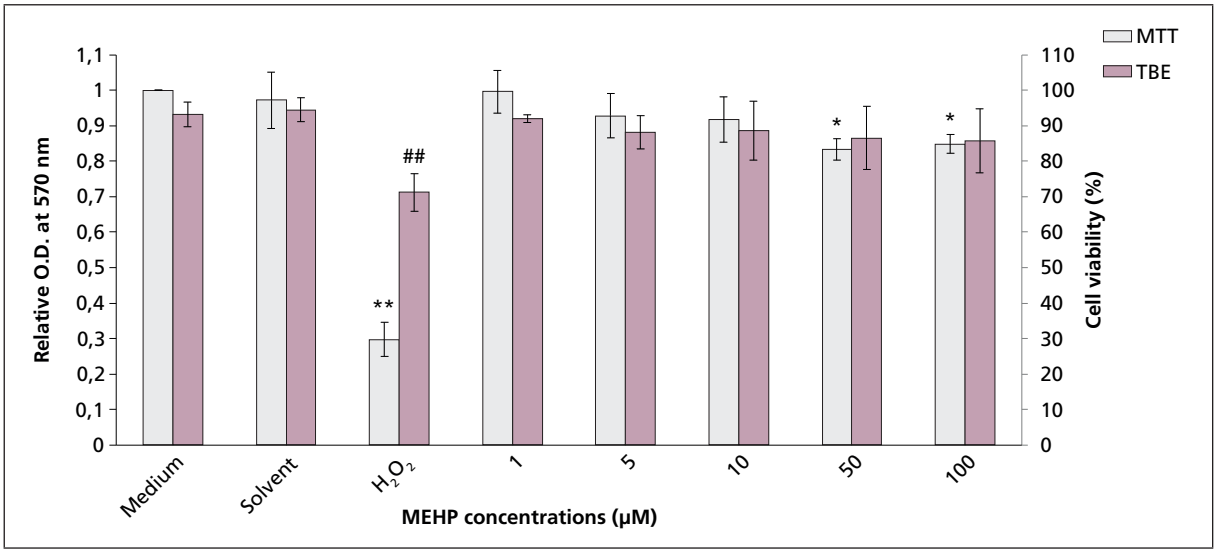

Figure 1

Cell viability determined through MTT and TBE assays in DLEC cell line exposed to MEHP. Results of MTT are displayed as a mean of the optical density (570 nm). MEHP treatment O.D. values were normalized to the solvent and are shown as mean ± SD of two experiments. Results of TBE are presented, at each treatment level, as the percent of viable cells out of the total cells and are displayed as mean ± SD of two experiments. One-way ANOVA significance: *$p<0.05$ MEHP treated *vs* solvent; **$p<0.01$ H_2O_2 vs untreated control (medium); ##$p<0.01$ H_2O_2 vs untreated control (medium).

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TBE: Trypan Blue Exclusion; DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylhexyl) phthalate; O.D.: Optical Density; SD: Standard Deviation.

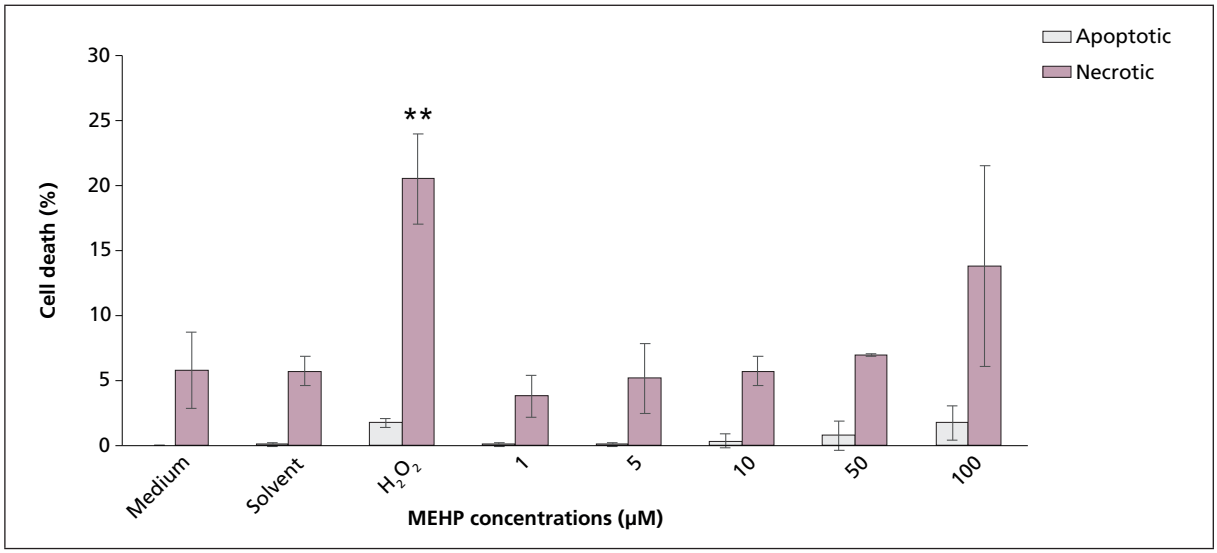

Figure 2
Percentage of apoptosis and necrosis in MEHP-treated DLEC cells. For each treatment results are shown as means ± SD of two independent experiments. One-way ANOVA significance: **$p<0.01$ H_2O_2 *vs* untreated control (medium).
MEHP: Mono-(2-ethylhexyl) phthalate; DLEC: European sea bass embryonic cell line.

trations, a dose-dependent reduction in DLEC viability was observed in both assays. In the MTT assay, survival rates of MEHP treatments significantly decrease, with respect to the solvent, only at the higher concentrations of 50 µM and 100 µM ($p<0.05$ and $p<0.0001$, respectively; F=189.4; DF=7), whereas in the TBE assay, decrease in survival resulted not to be statistically significant. In both assays, the solvent showed no effect on cell survival in DLEC. Conversely, treatment with H_2O_2 decreased cell survival to 30% ($p<0.0001$; F=3.409; DF=7) and 71.2% ($p=0.0535$; F=3.409; DF=7), respectively in the MTT and TBE assays.

Figure 2 shows the apoptosis induction in DLEC cells treated with MEHP. No induction of necrotic and apoptotic cells was observed in the untreated control and solvent samples. In MEHP treatments, induction of both necrosis and apoptosis were not statistically significant in comparison to the solvent. Conversely, with respect to the untreated control, in H_2O_2 treatment a statistically-significant induction of necrotic cells ($p=0.0001$; F=21; DF=7) and no increase in apoptotic cells were detected.

Comet assay

Table 1 illustrates the results of the mean TI values representing the induction of primary DNA damage in DLEC cells treated with MEHP. A TI of 5.63 was detected in the untreated control, while an increase in the TI value was observed in the solvent (TI=6.45), which was albeit not significant when compared to the untreated control. When compared to the solvent sample, treatment with MEHP did not induce any increase in TI values. The frequency of DNA damage was significantly increased by the H_2O_2 treatment (TI=30.50; $p<0.01$).

CBMN assay

Table 2 shows the induction of chromosomal damage as measured by CBMN assay. In binucleated cells, no difference in both the frequency of MN and CBPI values was observed in the solvent sample when compared to the untreated control. Conversely, treatment with MEHP revealed a dose-dependent and statistically-significant ($p<0.01$) increase in the yield of MN per cell at all MEHP concentrations with respect to the solvent. Moreover, a cytotoxic effect was detected as a decrease of CBPI values ($p<0.01$) and an increase in the percentage of cytostasis. Similarly, a statistically-significant increase in the frequency of MN ($p<0.01$) and a cytotoxic effect ($p<0.01$) were detected in H_2O_2 treatment in comparison to the untreated control.

As a further end-point to distinguish between a clas-

Table 1
Tail intensity (%) values obtained by the Comet assay in DLEC cell line treated for 24 h with MEHP. Data are presented as means ± SD of two independent experiments

Cell Line	Medium	Solvent	H_2O_2	MEHP concentrations (µM)				
				1	5	10	50	100
DLEC	5.63±1.14	6.45±1.44	30.50±5.70 §§	7.05±0.66	6.59±0.80	6.37±1.50	6.23±1.20	5.31±0.70

Significance of Student's *t*-test (ts): §§ $p\leq0.01$ H_2O_2 *vs* untreated control (medium). SD: standard deviation.
DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylexyl) phthalate.

Table 2
Induction of micronuclei (MN) in binucleated and mononucleated cells, cytokinesis block proliferation index (CBPI) and % of cytostasis in DLEC cell line treated for 24 h with MEHP and harvested after 48 h of cyto-B. Data are presented as means ± SE of two independent experiments

Treatment	MN/1000 BN±SE	ts	CBPI±SE	χ_2	% Cytostasis±SE	MN/1000 Mono±SE	ts
Medium	12.0±0.05		1.36±0.0003		0±0.00	10.3±0.06	
Solvent	13.2±0.08	NS	1.36±0.0006	NS	0±0.01	11.3±0.09	NS
H_2O_2	35.8±0.05	§§	1.26±0.0001	§§	26.3±0.07	15.2±0.01	§
0.5 µM	20.7±0.08	**	1.32±0.0002	**	12.6±0.11	22.0±0.09	**
1 µM	25.3±0.03	**	1.31±0.0001	**	13.6±0.16	28.3±0.05	**
5 µM	32.8±0.20	**	1.28±0.0002	**	22.3±0.12	40.3±0.05	**
10 µM	42.7±0.26	**	1.29±0.0005	**	19.4±0.02	50.7±0.11	**

Significance of *t*-Student test (ts) and Chi-squared test (χ_2): NS: not significant; **$p \leq 0.01$ MEHP treated *vs* solvent; $p \leq 0.05$ and §§$p \leq 0.01$ H_2O_2 *vs* untreated control (medium). SE: standard error.
DLEC: European sea bass embryonic cell line; MEHP: Mono-(2-ethylexyl) phthalate.

togenic or aneugenic effect of MEHP, the analysis of MN was performed in mononucleated cells as well. In the solvent sample, no difference in the frequency of MN was observed. With regard to MEHP treatments, a statistically-significant dose-dependent ($p<0.01$) increase in the frequency of MN, when compared to the solvent, was detected. When compared to the untreated control, H_2O_2 treatment caused a statistically significant ($p<0.05$) increase in the yield of MN, albeit lower with respect to MEHP.

DISCUSSION

The presence of phthalates in the marine environment has aroused great concern for aquatic organisms, due to the growing threat posed by plastic marine litter. Currently, it is unclear whether PAEs bioaccumulate and/or biomagnify through the trophic chain, and whether their metabolites may exhibit higher toxicity than their precursors. However, recent studies have shown that PAE metabolites, such as monobutyl phthalate, are easily stored in fat and biological tissues, reflecting a continuous and, thus, chronic exposure to living organisms [3, 5, 8]. Therefore, the assessment of their toxicity cannot be ignored. MEHP, as a DEHP primary metabolite, is one of the most studied pollutants since it is responsible for many of the effects of its parent compound [31]. For many organisms, including humans, MEHP toxic effects have been widely evaluated both *in vivo* and *in vitro*. Conversely, the *in vitro* toxicity of MEHP in cells deriving from marine fishes has been poorly investigated. Therefore, in the present work, the potential cytotoxic and genotoxic response to MEHP treatment on the European sea bass embryonic cell line, DLEC, has been analysed.

The cytotoxicity of MEHP, evaluated through the MTT and TBE assays, resulted in a significant decrease in cell viability only at the highest concentrations in the MTT assay. Given these results, MEHP displayed a minor cytotoxic effect with respect to DEHP in the DLEC cell line [20]. The variability observed in H_2O_2 cell survival results could probably be due to a different sensitivity of the MTT and TBE assays [32]. Cytotoxicity results are also sustained by a not significant induction of apoptosis and necrosis at all tested concentrations of this metabolite. Since the DLEC cell line lack of metabolic activation [15] the results suggest a direct cytotoxic effect of MEHP. In literature, large differences in sensitivity to MEHP and in its resulting cytotoxicity have been noted in both human and rodent cell lines [31]. Some *in vitro* studies have reported a variety of evidence of cytotoxicity starting from low concentrations of MEHP. For example, Erkekoğlu and collaborators [33, 34] reported a decrease in cell viability within 24 h, in MA-10 (mouse Leydig tumour) and LNCaP (human prostatic cancer) cell lines starting from 3 µM of MEHP treatment. On the other hand, other studies detected scarce cytotoxic effects of MEHP on rodent or human cell lines, if not at higher concentrations. In GCs (rat ovarian granulosa) and HepG2 (human liver) cell lines, a significant decrease in cell viability at 50 µM MEHP has been reported [14, 35], whereas HRT-8/SVneo (human placenta) cell lines were more resistant with a decrease in cell viability at a MEHP concentration of 180 µM [13]. The decreased viability of both HepG2 and HRT-8/SVneo was associated with an increase in apoptotic cells starting from 100 µM MEHP [13, 35]. Great variability in MEHP toxicity ranges can also occur within the same cell line. Indeed, other studies on the MA-10 cell line reported significantly-different results compared to Erkekoğlu and collaborators [33, 34], by finding a cytotoxic response starting at 300 µM [16] or at even higher concentrations, such as 1 to 3 mM of MEHP [15, 36], coupled with occasional encounters of apoptotic cell bodies in all MEHP tested treatments [36]. The reason for these discrepancies might lie in several factors: differences in the experimental designs, cell culturing conditions, cell density/number, cell source, purity of the MEHP, employment of secondary compounds [34], as well as the different responses of the diverse cell lines to this metabolite.

MEHP genotoxicity was assessed by both the Comet and the CBMN assays. The Comet assay is a sensitive test able to identify DNA strand breaks typically induced by clastogenic agents, while CBMN detects

both clastogenic and aneugenic effects, the latter being the induction of chromosomal loss events without the induction of DNA strand breaks. The results of the Comet assay do not suggest a genotoxic effect of this phthalate in DLEC cells. Indeed, the level of primary DNA damage, in terms of strand breaks revealed by the Comet assay, was not increased by MEHP, while H_2O_2, a clastogenic agent, caused a high and significant induction of DNA strand breaks. This outcome suggests that MEHP does not have a clastogenic effect in the DLEC cell line. Conversely, in the CBMN assay, MEHP induced a dose-dependent enhancement of MN, not only in the binucleated but also in the mononucleated cells, strongly suggesting an aneugenic action of this compound in the DLEC cell line. As reported in the literature, the possible cellular targets of MEHP triggering chromosome malsegregation could be the organization of the meiotic spindle and the assembly of actin [37]. Moreover, the same authors reported altered 5mC and H3K4me2 levels and a significant elevation of oxidative stress after MEHP treatment; it was recently suggested that the latter might have a role in chromosome alignment [38]. The genotoxic effects of DEHP and MEHP have been investigated in a number of different tissues and with various genotoxicity assays [5, 31]. MEHP genotoxic potential has been investigated in several studies by means of the Comet assay. For instance, Erkekoğlu and collaborators [33, 34] reported high levels of DNA damage associated with an increase in both Tail Moment and Tail Intensity by several folds at very low concentrations of MEHP (3 µM). Other authors detected an enhancement of DNA migration only at higher concentrations of MEHP [17, 39] and also a relationship between urinary concentrations of phthalate metabolites, including MEHP, and sperm DNA damage in humans [40]. However, to our current knowledge, no studies have been conducted on the induction of micronuclei by MEHP. The micronucleus assay is a methodology that makes it possible to obtain a measure of both chromosome breaks and whole chromosome loss [28]. With the CBMN assay, it is possible to detect between 60% and 90% of acentric fragments and, in combination with kinetochore/centromere detection or other genotoxicity assays (e.g. Comet assay), it is an optimal procedure for measuring whole chromosome loss events [28]. Moreover, scoring MN in mononucleated cells could be a further end-point able to distinguish agents with clastogenic action from aneugenic ones [41]. Indeed, Elhajouji and collaborators [41] first, and later Kirkland [42], demonstrated that increasing MN in mononucleated cells is a clear and sensitive index for detection of aneugenic compounds. Therefore, given the results of both the Comet and CBMN assays, it can be hypothesized that the dose-dependent increase in the frequency of MN caused by MEHP treatments on the DLEC cell line could represent chromosomal loss events rather than chromosomal breaks. Thus, this is the first experimental evidence of an aneugenic effect of MEHP.

In the present study, we demonstrated that MEHP pose a great risk for the European sea bass; since MEHP caused genomic instability to DLEC cell line it is possible that it may cause even more harm under natural condition, where this contaminant can accumulate in fish tissues and have a direct effect on target organs, such as the liver [43, 44]. In a recent study, Barboza and colleagues [7] have detected the presence of microplastic in the gastrointestinal tract, gills and also in the dorsal muscle of 150 commercial fishes (42% in *D. labrax*, n=50). The presence of microplastics in commercially important fish tissues may presents a risk to human health, due to the potential transfer of microplastics to humans, but also the potential toxicity of contaminants associated to plastic items [7, 18]. In humans, it is well known that phthalates in general can cause the disruption of endocrine function [18] and sperm DNA damage [40], however, information on the transfer of these pollutants to humans through the diet is still poorly investigated [18]. Since different studies showed that both wild and aquaculture fishes can bioaccumulate plastic additives, such as bisphenols and phthalates, in their tissues [43, 44], it is important to increase our knowledge on human absorption, distribution and metabolism of MEHP through fish ingestion, information indispensable for human health risk assessment.

CONCLUSION

The current study highlights a difference between the cytotoxic and genotoxic effects of DEHP and MEHP on the European sea bass embryonic cell line. In our previous study [20], we found that the cytotoxicity of DEHP was much higher compared to that caused by MEHP, its primary metabolite. Even the induction of apoptosis and necrosis was significantly higher after treatment with the precursor compound when compared to MEHP as well as the induction of MN. Thus, MEHP should probably be considered even more harmful than its precursor, because it induces genomic instability in the DLEC cell line at lower concentrations without triggering cell death. To conclude, this study underlines that MEHP, which is ubiquitous to the marine ecosystem as its parent compound, is an even more hazardous pollutant for the European sea bass, as well as for other marine organisms, and may represent an even greater risk for human health.

Authors' contributions

CM contributed conducting both cytotoxicity and genotoxicity assays, drawing Figures and Tables, performing data analysis, statistical analysis, writing the first draft and revising the final draft of the manuscript; SF contributed designing the experimental work, collaborating in both cytotoxicity and genotoxicity assays, performing data and statistical analyses and revising the final draft of the manuscript; GG contributed revising the final draft and bibliography of the manuscript; AC contributed collaborating in both cytotoxicity and genotoxicity assays; RM contributed to the conception and design of the work, interpretation of the data and revising the final draft of the manuscript; DA contributed to the conception and design of the work, interpretation of the data and revising the first draft of the manuscript; all the Authors approved the final version of the manuscript to be published.

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Conflict of interest statement

None

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