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# Effects of acute exposures to mecoprop, mecoprop-p and their biodegradation product (2-MCP) on the larval stages of the Pacific oyster, *Crassostrea gigas*

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ABSTRACT

Studies have shown that pesticides are sometimes detected at rather high levels in seawater and it has been suggested that these chemical compounds could act as additional stress factor for oysters cultured in coastal environments. The effects of pesticides on marine molluscs could be particularly harmful in the early stages which correspond to critical life stages. This study aimed to assess the effects of mecoprop, mecoprop-p and their degradation compound 2-methyl-4-chlorophenol on two larval stages of *Crassostrea gigas*. Embryotoxic effects were assessed on veliger larvae after 36 h exposures, and both percentages of normal larvae and types of abnormalities were taken into account. The effects of the three substances were evaluated on 21-day-old pediveliger larvae by calculating metamorphosis rates after 24 h exposures. The results of the embryotoxicity assay indicated that 2-methyl-4-chlorophenol was more toxic ($EC_{50}$: 10.81 mg $L^{-1}$) than its parent compounds ($EC_{50}$ mecoprop: 42.55 mg $L^{-1}$; $EC_{50}$ mecoprop-p: 78.85 mg $L^{-1}$). Mecoprop in particular injured shell formation with an increase of shell abnormalities following herbicide concentrations. The active substances were not toxic to metamorphosis processes, but 2-MCP was revealed to be more toxic to the success of metamorphosis ($EC_{50}$: 7.20 mg $L^{-1}$) than to embryo-larval development. However, the toxic concentrations were several orders of magnitude higher than environmental concentrations.



## 1. Introduction

Mecoprop (MCPP) is a chlorophenoxy herbicide used for broad-leaved weeds in cereal crops, grasslands and lawns, and it acts as a synthetic auxin. This is a racemic mixture containing equal amounts of two different stereoisomers. Only the (R)-(+) isomer (D form) is herbicidally active, with the (S)-(−) isomer holding no growth-promoting herbicidal activity (Fletcher et al., 1995). Mecoprop-p (MCPP-P) is a formulation containing >93% of the active (R)-(+) enantiomer. MCPP and MCPP-P are highly water soluble (20 °C) with values of 734 and 869 mg $L^{-1}$, respectively (PAN, 2013). For these highly polar substances, the low values of both log $K_{ow}$ (−0.19 to 0.02) and $K_{oc}$ (31) indicate that they are virtually non-bioaccumulative and they do not sorb appreciably to soil constituents (Tomlin, 1997; PPDB, 2013). They can leach easily from soils and are routinely detected in both surface waters and ground waters (Harrison et al., 2003). In Ontario streams, the enantiomer fractions (EF = R(+)/[R(+) + S(−)]) > 0.5 of mecoprop in stream water samples during 2006–2007 were related to the replacement of racemic mecoprop by single (+) enantiomer mecoprop-p after 2004 (Kurt-Karakus et al., 2010). Vink and his collaborators (1997), investigating pesticide biotransformation in 11 different surface waters located in a polder area, have reported a longer half-life for mecoprop ($DT_{50}$: 3-1400 days) compared to three other pesticides (including MCPA: 2-347 days). Finally, MCPP and MCPP-P are considered non-persistent in soils but very persistent in water (PPDB, 2013). 2-MCP is a degradation product of different parent pesticides including MCPP and MCPP-P (Oh and Tuovinen, 1991). Compared to the parent pesticides, data about its fate in the environment or its effects on ecotoxicological models are less available for this chemical transformation product. According to the Pesticide Properties DataBase (PPDB, 2013), 2-MCP is highly water-soluble and slightly bio-accumulates in aquatic organisms.

Littoral ecosystems including shellfish farming areas can be impacted by various contaminants originating primarily from terrestrial inputs (Auby et al., 2007; Buisson et al., 2008; Burgeot

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et al., 2008). Among pollutants, pesticides can enter aquatic systems via inputs from accidental or controlled sources (urban and industrial discharges) and from diffuse sources originating from domestic and agricultural activities (Akcha et al., 2012). Pesticides and especially herbicides are listed because of their high use in agricultural and non-agricultural activities. In Europe, pesticide consumption has declined by 50% compared to the average in the 1980s, but France remains the largest consumer of pesticides (Zhang et al., 2011). In France in 2007, fungicides and bactericides (36,919 tons) corresponded to the most-used pesticide types, followed by herbicides (26,808 tons) and finally insecticides (2101 tons) (Zhang et al., 2011; EUROSTAT, 2012). For herbicide use, the top 6 consumers are the USA, Mexico, Thailand, Colombia, Malaysia and France (FAO, 2013). In this context, the European legislation including the Water Framework Directive (2000/60/EC) and the Marine Strategy Framework Directive (2008/56/EC) aim to take all measures necessary to achieve or maintain a healthy ecological state of the coastal environment by 2015 and 2020, respectively. This objective includes a reduction of pesticide inputs into littoral areas.

In France, water quality is especially well surveyed in lotic and lentic ecosystems, and pesticides are analysed in a large network of localities. In 2007, the 15 most frequent molecules were herbicides, and among them, aryloxyalkanoic acids such as mecoprop and MCPA (which can both be degraded into 2-methyl-4-chlorophenol, 2-MCP) were quantified in 6.7% and 5.5% of the monitored sites (Commissariat Général au Développement Durable, 2010). Compared to glyphosate and its metabolite (AMPA), which are the most frequently detected molecules (in 22.2% and 43.1% of the sites, respectively), the frequency of mecoprop and MCPA quantification might be considered rather low. However, the presence of these molecules in French freshwater environments is not at all negligible. For example, in Normandy, which provides approximately 20% of French oyster production each year, aryloxyalkanoic acids were in the top 10 of the most-used herbicides; in 2006, the higher values recorded in rivers reached 0.084 $\mu g\,L^{-1}$ (mecoprop) and 0.091 $\mu g\,L^{-1}$ (MCPA) (Agence de l'Eau Seine Normandie, personal communication). In Switzerland, the quality goal for pesticides in surface waters (0.1 $\mu g\,L^{-1}$) was frequently exceeded for mecoprop (Gerecke et al., 2002). In this country, Wittmer and his collaborators (2010) have reported seasonal peak concentrations (e.g., 1.6 $\mu g\,L^{-1}$) driven by rain events from urban and agricultural areas; moreover, in the effluent of the studied storm sewer, these authors have indicated concentrations in mecoprop as high as 32 $\mu g\,L^{-1}$ from May to September and concluded that seasonal inputs cannot be neglected. The results of pesticide analyses strongly depend on the type of pesticide use (e.g., agricultural or non-agricultural uses) and the study period. The low levels of mecoprop frequently measured in the Danube River (maximum of 0.017 $\mu g\,L^{-1}$) and its major tributaries (maximum of 0.025 $\mu g\,L^{-1}$) have been imputed to the period of the survey, which was conducted in August and September (2007), an atypical application period for this pesticide (Loos et al., 2010). In the nine stations studied in the region of Barcelona (Spain), average and maximum concentrations of mecoprop were higher in April (0.006 and 0.025 $\mu g\,L^{-1}$, respectively) than in October (2004) (0.004 and 0.007 $\mu g\,L^{-1}$, respectively) (Kuster et al., 2008); a similar result was obtained by these authors for MCPA, but for this herbicide, all of the values were higher (e.g., maximum concentrations of 1.286 $\mu g\,L^{-1}$ in April and 0.067 $\mu g\,L^{-1}$ in October). In Ontario streams in 2006–2007, mecoprop was detected in 53% of the monitored locations with a concentration range of 0.0005–0.829 $\mu g\,L^{-1}$ and a mean of 0.062 $\mu g\,L^{-1}$ ($\pm$0.144) (Kurt-Karakus et al., 2010). The mass of mecoprop discharged in agricultural drainage waters directly onto salt marsh has been measured by Fletcher et al. (1995) and reached a value of 25.8 $\mu g\,L^{-1}$. The concentrations of pesticides are generally less monitored in estuarine and coastal waters than in freshwaters. Nevertheless, these hydrosytems are not spared (Scott et al., 2002; Arnold et al., 2004; Auby et al., 2007; Burgeot et al., 2008; Buisson et al., 2008). Of the 21 molecules surveyed in an oyster farming area (Marennes-Oléron), four pesticides including mecoprop were detected in seawater (Gagnaire, 2005), and there has been growing concern over the adverse effects of these compounds in non-target aquatic organisms.

Among the rare ecotoxicological data, the concentration leading to the death of 50% ($LC_{50}$) of a fish population exposed for 96 h can be cited for three types of substances: 2.3 $mg\,L^{-1}$ in *Lepomis macrochirus* exposed to 2-MCP; >100 $mg\,L^{-1}$ in an "unknown fish" species exposed to MCPP-P and 240 $mg\,L^{-1}$ in *Oncorhynchus mykiss* exposed to MCPP (PPDB, 2013). These values have to be considered with caution because they were recorded in different species, but they suggest a higher toxicity for the degradation product (PPDB, 2013). This conclusion is reinforced by the results of the classical ecotoxicological test performed in *Daphnia magna* for 48 h and provided by the same database (2013): $EC_{50}$ values of 0.29, 91 and >200 $mg\,L^{-1}$, respectively, for 2-MCP, MCPP-P and, MCPP which induces a low effect in the crustacean species. Low effects of mecoprop have also been reported by Nitschke et al. (1999) in *D. magna* (no immobilisation up to a concentration of 100 $mg\,L^{-1}$), in *Scenedesmus subspicatus* (no inhibition of algal growth up to 180 $mg\,L^{-1}$) and in the aquatic plant, *Lemna minor* ($EC_{50}$ values of 6.00 $mg\,L^{-1}$). For the marine copepod *Nitocra spinipes*, a value of $LC_{50}$ of 87,000 $\mu g\,L^{-1}$ after 96 h of exposure to MCPP has been calculated by Linden et al. (1979). Apart from these data provided by regulation models in aquatic ecotoxicology, the literature about the effects of MCPP (and MCPP-P) is poorly documented. Few studies have reported the effects of mecoprop alone on the physiology of mammals, including humans (e.g., Hooghe et al., 2000). The impacts of a mixture of five herbicides including mecoprop have been explored on the molecular and physiological responses of the European flounder, *Platichthys flesus* (Evrard et al., 2010). Greco and her collaborators (2011) have reported the combined impact of temperature and a pesticide mixture (mecoprop + 2,4-D + dicamba) on a battery of physiological biomarkers in *Mya arenaria*. A study by Bushek and his collaborators (2007) indirectly concerned oysters, as it assessed the effects of a commercial formulation including 10.6% mecoprop on the proliferation of the parasitic oyster pathogen *Perkinsus marinus*. To our knowledge, only one study has investigated the effects of mecoprop alone on *Crassostrea gigas*. Indeed, His and Seaman (1993) investigated the effects of 12 pesticides including mecoprop on veliger larvae from fertilisation to nine days.

Pollutant run-off into the ocean represents a potential threat to marine organisms, especially bivalves living in coastal environments (Renault, 2011). In this context, bivalve molluscs such as mussels and oysters have been postulated as ideal indicator organisms because of their wide geographical distribution, sedentary lifestyle and sensitivity to environmental pollutants. They are thus used in biomonitoring programs such as the American program Mussel Watch and the French program RNO/ROOCH (Goldberg, 1986; Cantillo, 1998). Moreover, bivalve farming corresponds to an important economic activity in many countries, including France, which is the leading producer of oysters in Europe and the fourth in the world. However, French oyster basins sporadically experience high summer mortalities (>30%) that have important socioeconomic consequences (Royer et al., 2007; Samain, 2007). These mortality outbreaks may result from multiple extrinsic and intrinsic factors including temperature variations, physiological stress and the presence of contaminants in the environment (Samain, 2007; Burgeot et al., 2008). Burgeot and his collaborators (2008) have reported that the oyster oxidative stress observed in June appeared to correspond with the peak of herbicide contamination in Marennes-Oléron (France).

There is a general assumption in ecotoxicology that early life stages (eggs, embryos and larvae) of invertebrates are more sensitive to xenobiotic agents than adults and therefore represent the critical life stages for ecotoxicological studies (Hutchinson et al., 1998; His et al., 1999; Mohammed, 2013). The early development stages of *C. gigas* are commonly used as sentinel organisms to assess the toxicity of a large variety of pollutants in marine bioassays (e.g., Geffard et al., 2002; Lyons et al., 2002; Poirier et al., 2007; Mai et al., 2012; Mottier et al., 2013) because of their relatively high sensitivity. By comparison, the metamorphosis success of bivalve larvae is rarely used as a biological endpoint for the monitoring of pollution in ecotoxicology (His et al., 1997; Mottier et al., 2013).

Regarding the paucity of existing ecotoxicological data relative to MCPP, MCPP-P and 2-MCP, the present study aimed to compare the toxicity of these three substances in a single species: the Pacific oyster, *C. gigas*. In the context of European legislation that essentially concerns parent pesticides, it is particularly interesting to assess the toxicity of degradation products such as 2-MCP, which might be more toxic according to data provided by model organisms classically used in ecotoxicology. The second objective of the study was to assess the usefulness of both embryo-larval toxicity and metamorphosis as toxicity biomarkers in marine ecotoxicology.

## 2. Materials and methods

### 2.1. Chemical compounds

Mecoprop (MCPP; [2(4-chloro-2-methylphenoxy) propanoic acid]; $C_{10}H_{11}ClO_3$) is a mixture of two stereoisomers in an equal proportion and acts as a selective, hormone-type phenoxy herbicide (CAS No: 7085-19-0; 99.5% purity). Mecoprop-p (MCPP-P) (CAS No: 16484-77-8) is a formulation containing the (R)-(+) enantiomer with 99% purity. 2-Methyl-4-chlorophenol, abbreviated as 2-MCP ($C_7H_7ClO$) (CAS No: 1570-64-5; 99% purity), is a degradation product that could originate from different parent pesticides such as MCPA ($C_9H_9ClO_3$), MCPA-thioethyl ($C_{11}H_{13}ClO_2S$), Mecoprop and Mecoprop-p. All of the pesticides used in this study were provided by Dr. Ehrenstorfer GmbH® (Augsburg, Germany). The herbicide solutions were prepared with natural open sea water sterilised on a 0.22 μm membrane (Steritop® Millipore). The concentration ranges were prepared from stock solutions at 500 mg $L^{-1}$.

For both endpoints, nominal concentrations corresponding to 0.1, 100 and 10,000 μg $L^{-1}$ of the chemicals (i.e., MCCP and MCCP-P) were verified (in duplicate) by ultraperformance liquid chromatography (UPLC) and MS-MS detection (in accordance with NF EN ISO 11 369) using a UPLC Acquity with TQD detector (Waters – Milford, MA, USA) and a column Waters Acquity BEH C18 – 2.1 mm × 150 mm, 1.7 μm. In accordance with the expected concentration, the samples were diluted or concentrated by solid phase extraction (Oasis – HLB 200 mg – Waters) before analysis. Moreover, the analyses were performed at the beginning and the end of the exposures to verify the variation in the tested concentrations during the period of the experiments. These analyses were performed once without embryos or larvae to avoid interaction between the physico-chemical and biological processes.

### 2.2. Embryotoxicity bioassay and experimental design

Conditioned oysters were purchased from the Guernsey Sea Farm Ltd. hatchery (Guernsey, UK). As previously described in Mottier et al. (2013), larvae were obtained using the standardised AFNOR procedure (AFNOR XP-T-906382) published in 2009. Briefly, male and female gametes were obtained by thermal stimulation (successive baths at 16 or 28 °C). The egg density of the selected female was determined with a Mallassez counting cell. Twenty minutes after fertilisation, the embryos were distributed into plastic pillboxes containing 25 mL natural sterilised sea water (0.22 μm, Steritop® Millipore) at a density of 60,000 $L^{-1}$ (corresponding to 1500 embryos per pillbox). After 36 h at 22 ± 1 °C without feeding, aeration or light, embryos or D-shaped larvae were fixed using 0.5 mL of an 8% formalin solution.

A minimum of 100 larvae was counted per replicate using an inverted binocular microscope at 400× magnification (Leica® DM IRB). Observations enabled the calculation of rates of abnormality and the discrimination of types of abnormalities; four categories could be distinguished: normal larvae, mantle abnormality alone (hypertrophies), shell abnormality (with/without additional mantle abnormality), late arrested development and early arrested development (when cells could be distinguished and counted) (Mottier et al., 2013). The results of embryo-larval development in exposed organisms were expressed as net percentages of normal development, $NPN_e$, adjusted for the controls (±SEM) (Mottier et al., 2013).

For the three molecules tested, two experiments corresponding to two couples of genitors were conducted and, for each experiment, herbicide concentrations were tested in triplicate. Consequently, the minimum number of individuals examined for each concentration was 600. Herbicide concentrations ranged from 0.1 to 100,000 μg $L^{-1}$ with a factor of 10× between two consecutive concentrations, this range being tightened between 10,000 and 100,000 μg $L^{-1}$ to precisely determine the $EC_{50}$ values (11 concentrations in total). $CuSO_4{\cdot}5H_2O$ (Alfa Aesar Gmbh®; Karlsruhe, Germany) was used as a positive control with concentrations ranging from 20 to 100 μg $L^{-1}$ (5 concentrations) according to the AFNOR procedure (AFNOR, 2009).

### 2.3. Metamorphosis bioassay and experimental design

The aim of this endpoint was to assess the metamorphosis rate of pediveliger larvae (ready for metamorphosis) exposed to herbicides. These pediveliger larvae (21 days old) were kindly provided by the SATMAR (Société ATlantique de MARiculture) hatchery (Barfleur, France). Larvae were exposed in multiwell plates (12-wells, NUNC®; Penfield, New York, USA) in a final volume of 1.5 mL natural sterilised seawater (0.22 μm, Steritop® Millipore). Larval density was set between 50 and 80 larvae per well. To promote metamorphosis, epinephrine (Sigma Aldrich®) was added at a final concentration of $10^{-4}$ M (Coon and Bonar, 1987). Experiments were conducted for 24 h at 22 °C without feeding, aeration or light.

After 24 h, exposed larvae were observed using an inverted binocular microscope at 100× magnification (Leica® DM IRB) to count dead larvae that exhibited tissue degradations and/or no movement. Following this first count, larvae were fixed using an 8% formalin solution. The metamorphosis rate was evaluated by counting metamorphosed versus non-metamorphosed larvae. A larva was considered metamorphosed when it presented an obvious loss of its velum, new shell growth and well-developed gills (Mottier et al., 2013). Dead larvae were very rarely observed, and metamorphosis rates were thus calculated by considering the percentages of non-metamorphosed versus metamorphosed ones. The results of the metamorphosis test in exposed organisms were expressed as net percentages of metamorphosis, $NPM_e$ (adjusted for the controls) (±SEM) (see Mottier et al., 2013).

Experiments were performed at least three times, and for each experiment, all herbicide concentrations were tested at least in triplicate. As for the embryotoxicity tests, the broad ranges of concentrations (between 0.1 and 100,000 μg $L^{-1}$) were tightened, from 10,000 μg $L^{-1}$ for MCCP and MCPP-P (3 additional concentrations) and from 1000 to 10,000 μg $L^{-1}$ for 2-MCP (8 additional concentrations).

**Table 1**
Pesticides concentrations (mean values in $\mu g\,L^{-1} \pm$ SEM) measured for both endpoints at the beginning of the experiment and after 24 h or 36 h of exposure to MCPP and MCPP-P. (NC = Nominal Concentrations, MC = Measured Concentrations, % = percentage of differences between NC and MC.).

| | | Embryotoxicity | | | | Metamorphosis rate | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | T0h | | T36h | | T0h | | T24h | |
| | NC | MC | % | MC | % | MC | % | MC | % |
| Mecoprop ($\mu g\,L^{-1}$) | 0.1 | 0.115 ± 0.005 | 15.5 | 0.099 ± 0.006 | 1.25 | 0.098 ± 0.009 | 2.25 | 0.105 ± 0.012 | 5.25 |
| | 100 | 89.85 ± 10.54 | 10.15 | 104.65 ± 0.92 | 4.65 | 97.7 ± 4.24 | 2.3 | 111.15 ± 5.59 | 11.15 |
| | 10,000 | 10,490 ± 975.81 | 4.9 | 9020 ± 254.56 | 9.8 | 9940 ± 509.12 | 0.6 | 9370 ± 947.52 | 6.3 |
| Mecoprop-p ($\mu g\,L^{-1}$) | 0.1 | 0.10 ± 0.01 | 2 | 0.101 ± 0.01 | 1.25 | 0.089 ± 0.01 | 11.5 | 0.084 ± 0.01 | 15.75 |
| | 100 | 79.4 ± 4.10 | 20.6 | 95.1 ± 7.07 | 4.9 | 74.65 ± 1.91 | 25.35 | 84.9 ± 5.66 | 15.1 |
| | 10,000 | 8760 ± 820.24 | 12.4 | 10,380 ± 282.84 | 3.8 | 8310 ± 240.42 | 16.9 | 7874 ± 545.89 | 21.26 |

### 2.4. Statistical analyses

Data about the chemical analyses at T0 versus T36h (embryotoxicity) or T24h (metamorphosis) were statistically compared using non-parametric Mann–Whitney tests. Because the metamorphosis data did not met the assumption of normality even with various transformations, they were analysed with a Kruskall & Wallis non-parametric test. Comparisons between concentrations were then performed with a modified Student Newman Keuls for non-parametric data (Scherrer, 1984). Embryotoxicity data were normalised if needed (arc-sinus transformation for MCPP data), and potential differences were tested using one-way ANOVAs. Differences among concentrations were analysed with Student Newman Keuls tests. The statistical analyses were performed using STATISTICA 8.0 software (Statsoft®, Tulsa, OK, USA). $EC_{10}$ and $EC_{50}$ values were computed with non-linear regressions (Hill equation) using Excel® macro REGTOX (Vindimian, 2012).

## 3. Results

### 3.1. Analyses of the tested molecules

At the beginning of both types of experiments (T0), the measured concentrations were slightly lower than the nominal ones except for the embryotoxicity tests at 0.1 and 10,000 $\mu g\,L^{-1}$ of MCPP (0.1155 and 10,490 $\mu g\,L^{-1}$, respectively) (Table 1). Nevertheless, the differences were generally not important and reached maxima of 20.6% and 25.35% at 100 $\mu g\,L^{-1}$ of MCCP-P for the embryotoxicity test and metamorphosis assay, respectively. The differences between nominal and measured concentrations did not appear to depend on the endpoint (embryotoxicity versus metamorphosis) despite the two types of experimental design.

For both tests, the measured concentrations at T36h (embryotoxicity) or T24h (metamorphosis) did not differ significantly from those recorded at T0 (Mann–Whitney tests, $0.24 < p < 1$) (Table 1). Consequently, it could be considered that organisms were exposed to constant concentrations during the whole experiments.

### 3.2. Embryotoxic effects of herbicides

All of the assays performed could be considered valid because they met the requirements of the AFNOR standardised procedure (2009). Fecundity levels were high, and the raw rates of normal larvae in controls were up to 80% for all of the experiments (from 80.61% ± 1.14 to 87.75% ± 2.36). Finally, the effects of the control contaminant ($CuSO_4{\cdot}5H_2O$) were in accordance with the expected concentration-response curves, and $EC_{50}$ ranged between 6 and 16 $\mu g\,L^{-1}$ $Cu^{2+}$ regardless of the experiment.

The three tested contaminants exhibited toxic effects on embryo-larval development. The 2-MCP contaminant was revealed to be the most toxic, with an $EC_{50}$ of 10,810.22 $\mu g\,L^{-1}$, whereas those recorded for MCPP and MCPP-P reached 42,553.55 $\mu g\,L^{-1}$ and 78,853.13, respectively (Table 2). The $NPN_e$ values were significantly affected from 10,000 $\mu g\,L^{-1}$ (68.25% ± 6.12); 40,000 $\mu g\,L^{-1}$ (52.68% ± 12.07) and 80,000 $\mu g\,L^{-1}$ (43.83% ± 19.8) for 2-MCP, MCPP and MCPP-P, respectively (Fig. 1A–C) (ANOVAs < 0.05; SNKs < 0.05). Furthermore, among the three contaminants, only 2-MCP and MCPP led to 0% of normal development for the concentrations of 20,000 and 100,000 $\mu g\,L^{-1}$, respectively, whereas 25.69% (±11.55) of normal larvae were observed at the highest concentration of MCPP-P (100,000 $\mu g\,L^{-1}$).

Whatever the tested chemical, results related to the types of abnormalities indicated that late arrested development was the predominant abnormality at high doses of contaminants (100,000 $\mu g\,L^{-1}$: 100% of embryos in MCPP; 98.34% in 2-MCP and 56.32% in MCPP-P) (Fig. 2). Moreover, MCPP appeared to specifically affect shell formation, with a proportion of shell abnormality reaching 29.75%, 44.08% and 44.36% at concentrations of 40,000, 60,000 and 80,000 $\mu g\,L^{-1}$, respectively (Fig. 2A). The shell deformations are illustrated in Fig. 3. Such a response could not be observed for MCPP-P or 2-MCP, which were associated with maximum rates of shell abnormality of 20.19% and 15.12%, respectively, and followed the same pattern with a concentration shift (Fig. 2B and C).

**Table 2**
Ecotoxicological parameters calculated for the embryotoxicity tests (rates of abnormalities in D-shaped larvae) and the rates of pediveliger larvae metamorphosis after 48 h exposures to 3 herbicide substances: mecoprop (MCPP), mecoprop-P (MCPP-P) and 4-chloro-2-methylphenol (2-MCP). Ecotoxicological parameters are given for nominal and corrected concentration. $EC_X$ = effective concentration (in $\mu g\,L^{-1}$) which induces an effect on *X*% of the population (10% or 50%). na = non-available data.

| Endpoints | Parameters | MCPP | MCPP-P | 2-MCP |
|---|---|---|---|---|
| Abnormality rates in D-shaped larvae (nominal concentrations) | $EC_{10}$ | 32,178.29 | 50,489.47 | 8873.72 |
| | $EC_{50}$ | 42,553.55 | 78,853.12 | 10,810.22 |
| Abnormality rates in D-shaped larvae (measured concentrations) | $EC_{10}$ | 26,655.49 | 51,361.05 | na |
| | $EC_{50}$ | 34,479.17 | 80,951.71 | na |
| Metamorphosis rates of pediveliger larvae (nominal concentrations) | $EC_{10}$ | >100,000 | >100,000 | 5603.40 |
| | $EC_{50}$ | >100,000 | >100,000 | 7199.79 |
| Metamorphosis rates of pediveliger larvae (measured concentrations) | $EC_{10}$ | >100,000 | >100,000 | na |
| | $EC_{50}$ | >100,000 | >100,000 | na |

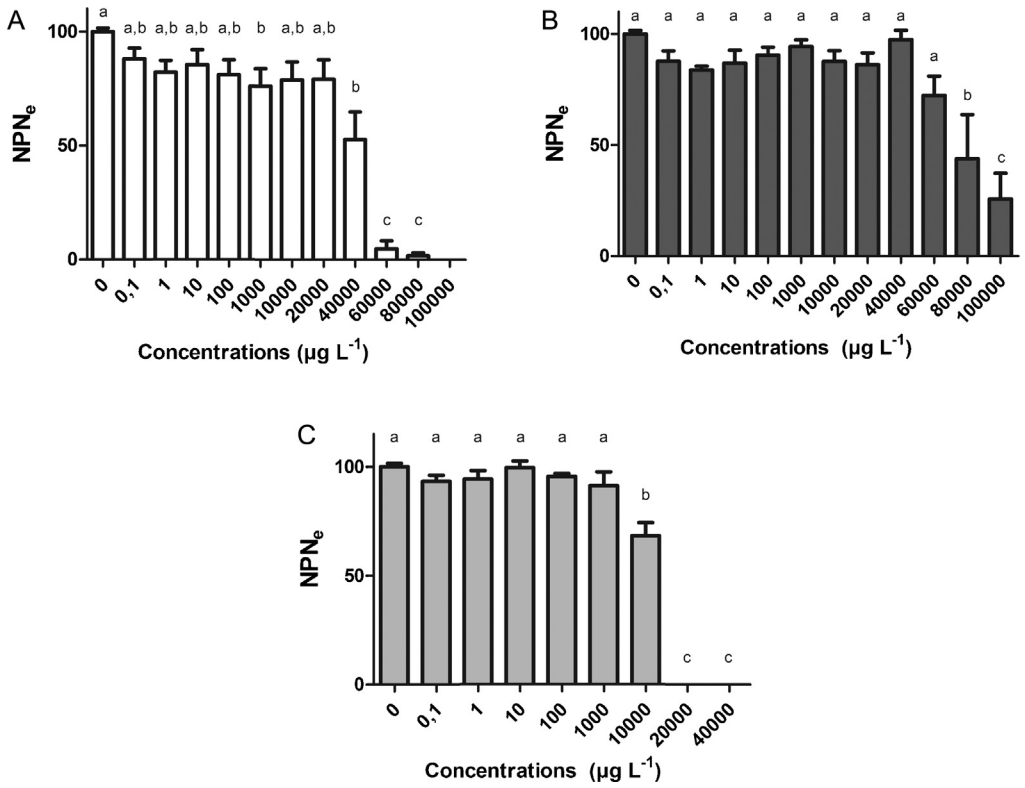


**Fig. 1.** Net percentages of normal development ($NPN_e$) (±SEM) in *C. gigas* embryo-larvae observed after 36 h of exposure to herbicides at concentrations ranging from 0.10 to 100,000 µg $L^{-1}$ for mecoprop (MCPP) (A), mecoprop-p (MCPP-P) (B) and 2-methyl-4-chlorophenol (2-MCP) (C). The concentrations that do not share a letter are significantly different.

### 3.3. Effects of herbicides on larval metamorphosis

With regard to all experiments, the raw mean metamorphosis rate was 96.93% (±0.72) for the controls. MCPP had no effect on the process of metamorphosis. In fact, metamorphosis rates ($NPM_e$) recorded for all of the tested concentrations of this herbicide ranged from 89.36% (±3.24; 100,000 µg $L^{-1}$) to 101.17% (±0.566; 10 µg $L^{-1}$), and no significant differences were observed (Kruskall & Wallis, $p > 0.05$) (Fig. 4A). The effects of MCPP-P on metamorphosis were also extremely limited. Rates of metamorphosis were up to 96.46% from 0 to 75,000 µg $L^{-1}$, and a slight but significant decrease was recorded at the highest concentration of 100,000 µg $L^{-1}$ (Kruskall & Wallis, $p < 0.05$; SNK, $p < 0.05$) (Fig. 4A). As the two herbicide molecules were practically non-toxic for the tested range of concentrations, it was thus not possible to compute $EC_x$ values.

Compared to the parent compounds, 2-MCP was revealed to be more toxic to the metamorphosis of *C. gigas* larvae. The $NPM_e$ recorded for the control did not differ significantly from those calculated for the concentrations from 0.1 to 4000 µg $L^{-1}$ ($NPM_e$ from 95.67% to 100.40%) (Fig. 4B). From the concentration of 6000 µg $L^{-1}$, the metamorphosis of larvae was significantly affected (88.04% ± 2.133) (Kruskall & Wallis, $p < 0.01$; SNK, $p < 0.05$), and a sharp decrease was recorded from 6500 µg $L^{-1}$ (70.54% ± 7.41) to 10,000 µg $L^{-1}$ (4.01% ± 1.04). The $EC_{10}$ and $EC_{50}$ computed with non-linear regressions applied to the data were 5603.4 and 7199.79 µg $L^{-1}$, respectively (Table 2).

## 4. Discussion

### 4.1. Concentrations of chemicals and their dynamics

For both endpoints, chemical analyses were performed to assess the potential changes in MCPP and MCPP-P concentrations during the experiments. The measured concentrations at the end of the experiment did not differ significantly from those recorded at the beginning of the assay. Unfortunately, 2-MCP could not be measured in this study and data about the features (e.g. hydrolysis) of pesticides including 2-MCP generally refer to freshwater environments (surface freshwaters showing different pH values) and not to seawaters. Nevertheless, it can be hypothesised that the fate of 2-MCP in our experimental structures did not differ meaningfully from those of MCPP and MCCP-P. In fact, like both of its parent compounds, 2-MCP is highly water-soluble; data about its half-life are scarce, but hydrolysis (and photolysis) are estimated to be negligible (OECD, 2013). In the same experimental structures as those used in the current study, Mottier et al. (2013) showed that the concentrations of glyphosate and AMPA did not change during embryotoxicity and metamorphosis bioassays, although they are reported as non-persistent molecules. Because 2-MCP belongs to the herbicide family of chlorophenols, which are well known for being difficult to remove from the environment and exhibiting a long half-life in water (Pera-Titus et al., 2004), it can be assumed that these concentrations were also constant during the whole experiments. For all three compounds, significant hydrolysis could thus be excluded, and photolysis could not occur because the experiments were carried out in the dark. MCPP and MCPP-P analyses were also conducted to verify the nominal concentrations by comparing them with measured ones. The differences were rather low, with the maximum differences observed for pediveliger larvae exposed to MCCP-P at T0 and T24h (17–18% in average); intermediate percentages (10–11%) were calculated for embryotoxicity at T0 for both herbicides, and the other differences did not exceed 7% (Table 1). The differences between nominal and measured concentrations appeared to be globally independent of both the molecules and the endpoints tested and were most likely due to variation in handling. To take these differences into account, the values of $EC_{50}$

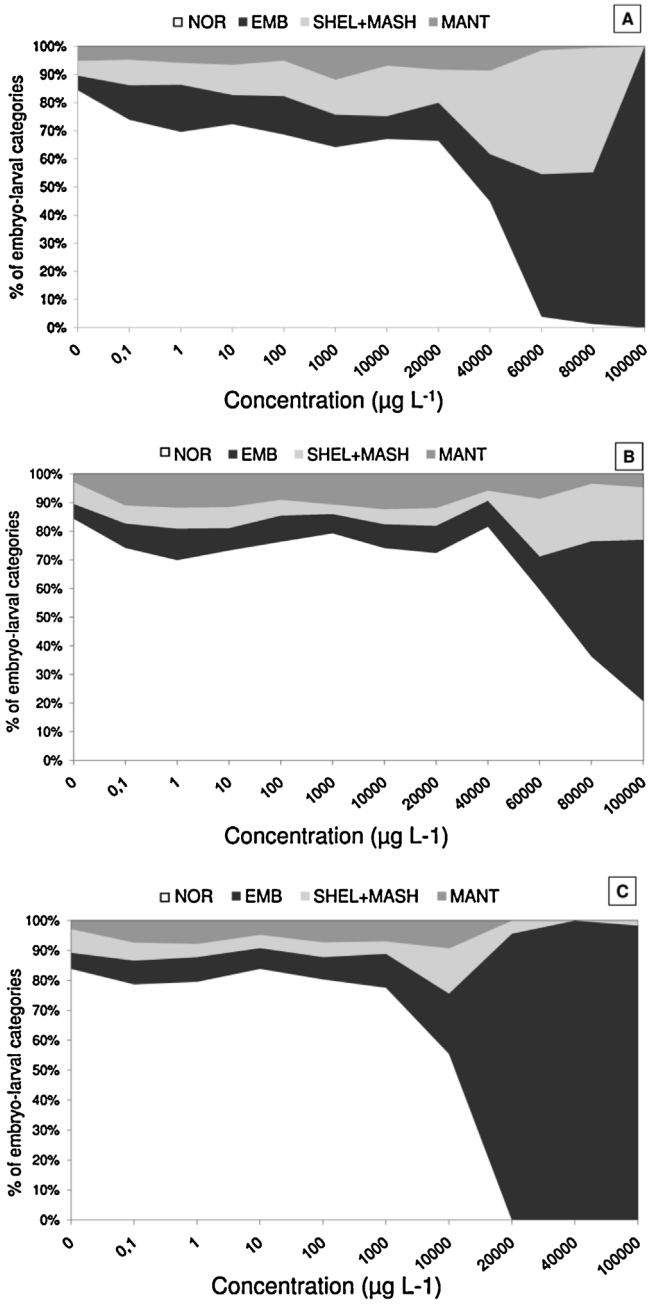


**Fig. 2.** Occurrence of the various types of abnormalities affecting embryo-larval development in *C. gigas* after 36 h of herbicide exposure in relation to the concentrations of three chemicals: mecoprop (MCPP) (A), mecoprop-p (MCPP-P) (B) and 2-methyl-4-chlorophenol (2-MCP) (C). NOR: normal D-shaped larvae; EMB: "old embryo"; SHEL + MASH: D-shaped larvae exhibiting shell and/or hinge abnormalities (with/without additional mantle abnormality); MANT: D-shaped larvae with a hypertrophied mantle.

in organisms exposed to MCPP and MCPP-P were recalculated using measured concentrations, but the values of this ecotoxicological parameter were within the same order of magnitude and did not change the conclusions.

### 4.2. Effects of mecoprop and herbicide mixtures including mecoprop on aquatic animals

With $EC_{50}$ values of 79–81 and 34–43 mg $L^{-1}$ (Table 2), MCCP-P and MCCP can be classified as "slightly toxic" (US EPA Toxicity Classification) or "harmful" (European Toxicity Classification), both of those categories characterised by $EC_{50}$ values ranging from 10 to 100 mg $L^{-1}$ (Giesy et al., 2000). In the case of 2-MCP, the calculated $EC_{50}$ value was lower (11 mg $L^{-1}$), which placed this degradation compound in the same category but closer to the category of "moderately toxic" (US EPA Toxicity Classification) or "toxic" (European Toxicity Classification) (1 mg $L^{-1}$ < $EC_{50}$ < 10 mg $L^{-1}$). It is notable that the glyphosate by-product (AMPA) was less toxic for oyster larvae than the parent molecule (Mottier et al., 2013), whereas 2-MCP was revealed to be more toxic than both MCCP and MCCP-P. Such a conclusion should be highlighted because European regulation primarily concerns active substances, but in aquatic environments, the degradation products of pesticides have also to be considered, especially when they are more toxic than the active molecules or commercial formulations. For target organisms (cereal crops, grasslands and lawns), MCPP-P exhibits a higher herbicide activity because it is composed essentially of the active (R)-(+) enantiomer, whereas MCCP, which includes both enantiomers, is a less effective herbicide and consequently has been progressively replaced by MCCP-P from the 1980s. By contrast, in non-target organisms such as oyster larvae, exposure to MCCP led to the calculation of a lower $EC_{50}$ value and thus was revealed to be more toxic than MCCP-P; this result suggests a greater impact of the (S)-(−) isomer than the (R)-(+) enantiomer on *C. gigas* embryo-larval stages. These herbicides act on target organisms by disturbing the regulation of auxin production, but that type of phytohormone does not exist in animals, including oysters. Other modes of action thus have to be considered, but the identification of such an alternative mode is difficult, notably because little is known about herbicide toxicity on non-target organisms or the precise mechanisms occurring during embryo-larval development in *C. gigas*. In the current study, the herbicides induced serious damages in the young embryos preventing them from developing from different thresholds according to molecules (10,000–60,000 µg $L^{-1}$). At the highest concentration (100,000 µg $L^{-1}$), the primary (for MCCP-P) or the only (for MCCP and 2-MCP) abnormality type observed corresponded to arrested development at the embryo stage, which is the most severe type of damage, as individuals failed to reach the D-larva stage. The second most severe abnormality appeared to concern shell formation; relatively high rates of shell deformities (including mantle abnormalities) were recorded for concentrations from 60,000 and 40,000 µg $L^{-1}$ for MCPP-P and MCPP, respectively. In fact, rates of shell abnormalities could exceed 20% and peaked at 44.31% for the MCPP concentration of 80,000 µg $L^{-1}$. Such high rates were not calculated for *C. gigas* exposed to glyphosate-based herbicides because the frequency of shell abnormalities was generally low and reached maxima from 5.96% (0.1 µg $L^{-1}$ of Roundup Express) to 9.78% (0.1 µg $L^{-1}$ of AMPA) (Mottier et al., 2013). Moreover, the larvae exposed to glyphosate-based herbicides exhibited little shell deformation compared to the larvae submitted to mecoprop and especially to MCPP (Fig. 3); Mottier et al. (2013) wondered whether shell abnormalities (showing different degrees of severity) were viable or lethal. In the current study, the shell deformations observed were generally more severe, and it can be assumed that they might not allow the survival of the affected larvae. Although 2-MCP did not particularly induce shell abnormalities (maximum of 9.27% for the concentration of 10,000 µg $L^{-1}$), MCPP (and to lesser extent MCPP-P) at high concentrations (from 40,000 µg $L^{-1}$) appeared to act by disturbing shell formation. Nice and his collaborators (2000) have reported larval deformities in *C. gigas* larvae exposed to 4-nonylphenol, but these malformations appeared to be less drastic and essentially concerned the hinge, which was convex instead of right. These authors (2000) suggested a chemical interaction with shell development through the calcium metabolism of the larvae; i.e., calcium being possibly "mobilised for use elsewhere within the organism". Future works are needed to accurately investigate the biomineralisation processes in *C. gigas* larvae exposed

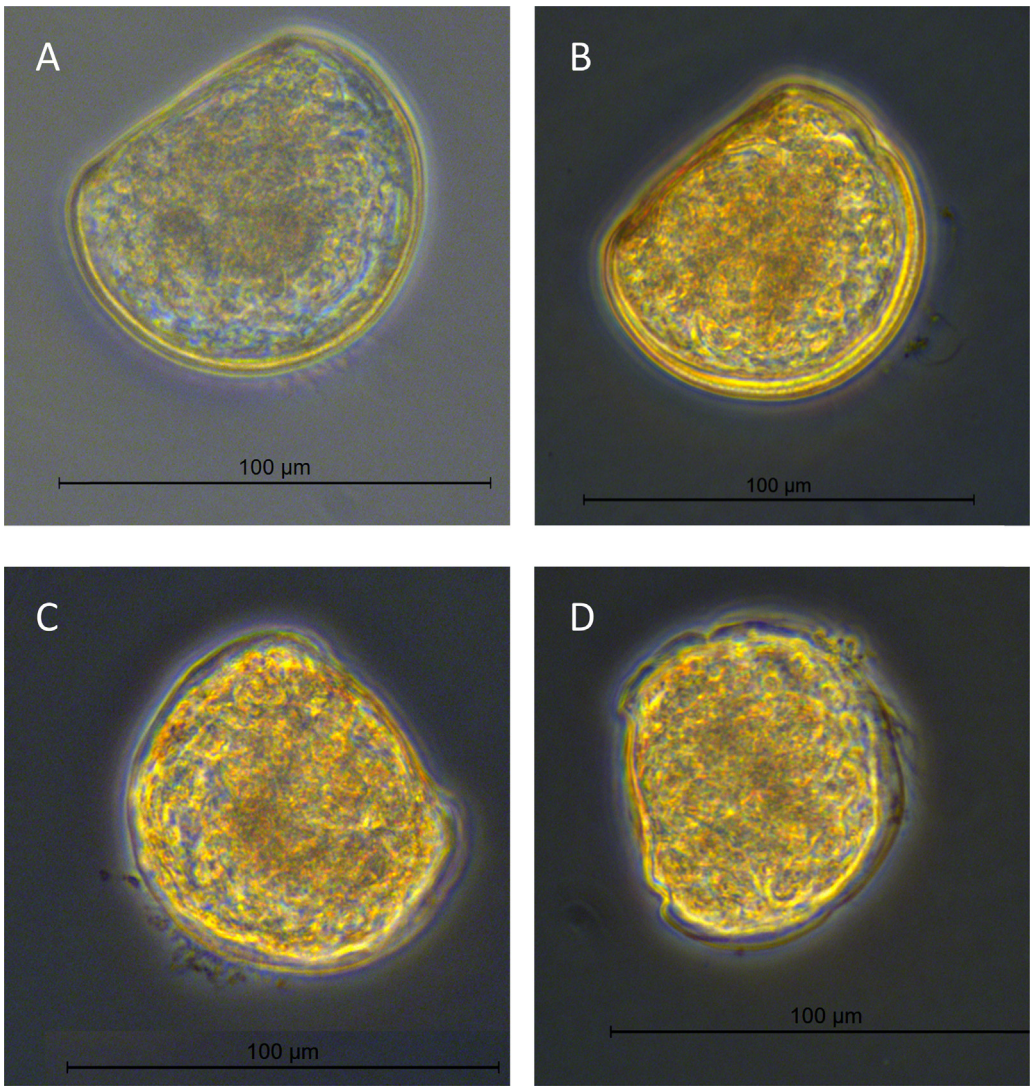


**Fig. 3.** Light microscopy images (400×) showing (A) a normal D-shaped veliger and (B–D) D-shaped veligers showing increasingly severe shell abnormalities.

to MCPP. These studies would be especially useful because there are few data about the effects of pollutants on the shell of marine molluscs, except the impact of tributyltin on the shell of adult individuals (e.g. Meng et al., 2005).

With regard to embryotoxicity, the three tested substances could be classified according an increasing gradient of toxicity as follows: MCPP-P, MCPP and 2-MCP. For the metamorphosis test, the degradation product was also revealed to be more toxic than MCPP and MCPP-P. For the two parent molecules, the rate of metamorphosis remained high even at the highest concentration (100,000 $\mu g\,L^{-1}$), and it was thus impossible to compute $EC_{50}$ values. However, at this highest concentration, the metamorphosis rate of pediveliger larvae exposed to MCPP-P (but not to MCPP) was slightly but significantly lower compared to the other concentrations. To definitely conclude about a possible difference in the toxicity of MCPP versus MCPP-P to the success of metamorphosis in *C. gigas*, further experiments (including concentrations higher than 100,000 $\mu g\,L^{-1}$) would be necessary but not very useful, as both substances can be considered "practically nontoxic" according to the US EPA Toxicity Classifications (Giesy et al., 2000). Compared to glyphosate and its by-product (AMPA) (Mottier et al., 2013), MCPP and MCPP-P exhibited similar results with $EC_{50}$ values higher than 100,000 $\mu g\,L^{-1}$. By contrast, the $EC_{50}$ value computed for pediveliger larvae exposed to 2-MCP was 7199 $\mu g\,L^{-1}$. This value was slightly higher but within the same order of magnitude as those calculated for Roundup Express (6366 $\mu g\,L^{-1}$) and Roundup Allées et Terrasses (6060 $\mu g\,L^{-1}$) by Mottier and his collaborators (2013). These three substances can be qualified as "moderately toxic" (US EPA Toxicity Classification) or "toxic" (European Toxicity Classification) (Giesy et al., 2000). Unfortunately, the mode of action of 2-MCP (and that of its parent compounds) on metamorphosis processes still remains unknown. For the metamorphosis test, the protocol involves the addition of $10^{-4}$ M epinephrine to induce metamorphosis in larvae placed in multiwall plates (which do not offer an optimal surface for larvae settlement). According to the present results for 2-MCP, it can be hypothesised that this molecule interacts negatively with epinephrine. In the presence of 2-MCP, pediveliger larvae did not die or exhibit apparent problems, but they were very active and swam and moved constantly.

His and Seaman (1993) compared the toxicity of 12 pesticides, including mecoprop, to the survival and growth of *C. gigas* embryo-larval stages from fertilisation to nine days (veliger stage). They concluded that there was a lethal effect of mecoprop on 50% of the studied larval population after nine days of exposure to a mecoprop concentration of 4200 $\mu g\,L^{-1}$. This relatively low value indicated a higher toxicity of mecoprop compared to the $EC_{50}$ values found in the current study for embryo-larval development and metamorphosis. His and Seaman's results are especially interesting

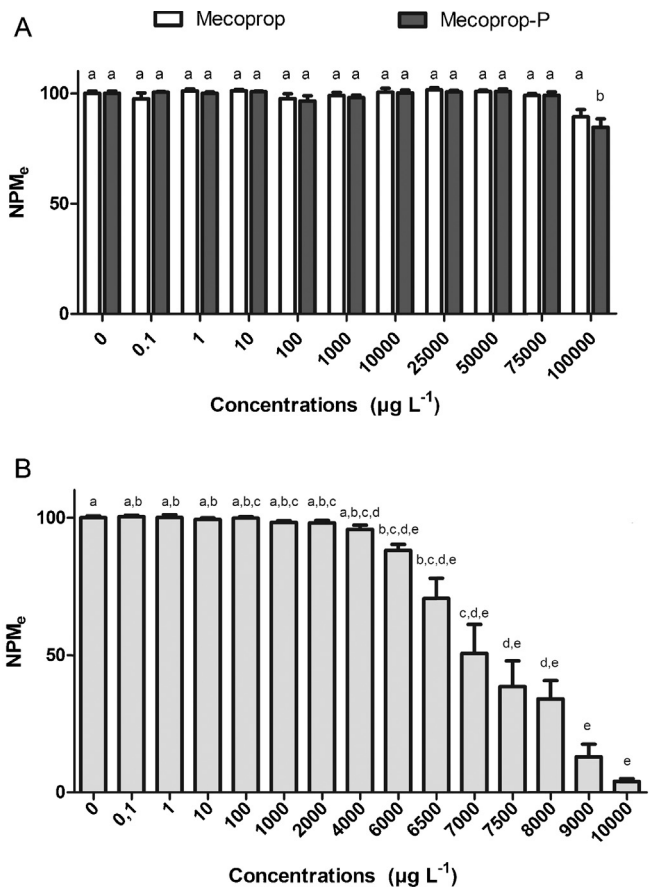


**Fig. 4.** Net percentages of metamorphosed larvae ($NPM_e$) (±SEM) observed after 24 h of exposure to herbicides at concentrations ranging from 0.1 to 100,000 µg $L^{-1}$ for glyphosate and AMPA (A) or 10,000 µg $L^{-1}$ for mecoprop (MCPP) and mecoprop-p (MCPP-P) (A) and 2-methyl-4-chlorophenol (2-MCP) (B). The concentrations that do not share a letter are significantly different.

because they demonstrated that longer exposures (9 days) could prove more injurious to oyster larvae than short exposures (24 or 36 h). Moreover, the $LC_{50}$ recorded by His and Seaman (1993) indicated that mecoprop was less toxic than isoproturon or lindane but more toxic than five other pesticides, including two insecticides and one molluscicide that are generally considered more toxic to animal organisms (parathion methyl, carbofuran and metaldehyde). With regards to shell growth for nine days, His and Seaman (1993) reported a 10% height reduction in response to exposure to a rather low concentration: 130 µg $L^{-1}$. In comparison with larval mortality, this endpoint indicated different results, as only one insecticide (parathion methyl) appeared to be more toxic than mecoprop, one molecule (lindane) produced the same $EC_{50}$ value and eight pesticides were less toxic according to this biomarker. Moreover, and quite surprisingly for a pesticide, His and Seaman (1993) suggested a hormesis effect at the lowest tested concentration (50 µg $L^{-1}$), for which slight additional growth was observed. Aside from this positive effect, the authors recorded inhibition of shell growth from 100 µg $L^{-1}$; this effect on the oyster shell can be linked with the shell deformities reported in the current study. In Pectinidae, specifically *Pecten maximus*, Larvor-Cario and her collaborators (2000) have demonstrated modifications in shell calcareous structures; these authors suggested a link with salinity decreases and also with two pesticides: diuron and mecoprop. In estuaries and coastal ecosystems, organisms are generally exposed to a mixture of various pollutants. Moreover, the effects of these contaminants are dependent on other abiotic parameters such as salinity or temperature. For example, soft-shell clams (*Mya arenaria*) exposed to a pesticide mixture composed of mecoprop, 2,4-D and dicamba and acclimated to 7 °C versus 18 °C exhibited different patterns of physiological responses (Greco et al., 2011); the authors concluded that increased temperature altered the ability of the sentinel species to respond to pesticide exposures. Using transcriptomic approaches, a significant effect of an herbicide mixture including mecoprop has been demonstrated on the liver in the European flounder *P. flesus* when exposed for 62 days; the methionine level, lipid transport and metabolism, immunity and respiratory chain were particularly affected (Evrard et al., 2010). Finally, mixtures of pesticides can also have an indirect positive effect on oyster populations by acting on their pathogens, such as the protozoan *P. marinus*, as demonstrated by Bushek and his collaborators (2007). During in vitro experiments, the proliferation of *P. marinus* was significantly inhibited by an herbicide mixture including 10.6% mecoprop (Weed-B-Gone®), but not at or below the manufacturer's recommended application rate.

### 4.3. Effects of various contaminants on the embryo-larval development in C. gigas

Comparisons between polluted sites or between pollutants are especially relevant if the same species is considered. The embryotoxicity of five types of pesticides have already been assessed in *C. gigas*: an insecticide (endosulfan; Wessel et al., 2007); a molluscicide (methiocarb; His et al., 1999); a fungicide (Opus®: epoxiconazole; Amara, 2012), an algaecide (Irgarol® substituting TBT in anti-fouling paints; Mai et al., 2012); and a higher number of herbicides: triazine herbicides (atrazine and simazine), dinoterb (Robert et al., 1986), metolachlor (Mai et al., 2012), diuron (Akcha et al., 2012) and finally glyphosate-based herbicides (His et al., 1999; Akcha et al., 2012; Mottier et al., 2013). The embryotoxicity test applied to *C. gigas* has also been used to estimate the effect of metals (Martin et al., 1981; His et al., 1999; Mamindy-Pajany et al., 2013), alkylphenols (Nice et al., 2000), nonylphenols (Amara, 2012), PAHs (Lyons et al., 2002; Wessel et al., 2007), endocrine disruptors (Wessel et al., 2007), fungal peptides (Poirier et al., 2007); and to test the effects of polluted sediments (Geffard et al., 2002; Cachot et al., 2006). A summary of the toxicity levels of these various compounds is given in Table 3. This table does not include data on copper ($CuSO_4{\cdot}5H_2O$), as that metal is generally used as a positive control, and $EC_{50}$ ranges from 8.47–12.43 µg $L^{-1}$ $Cu^{2+}$ (Mottier et al., 2013) to 22–40 µg $L^{-1}$ $Cu^{2+}$ (Poirier et al., 2007). Some of these studies have focused on relatively narrow concentration ranges, and the $EC_{50}$ could thus not be determined, whereas we tested a wide range of herbicide concentrations including both low (0.1 µg $L^{-1}$) and very high (up to 100 mg $L^{-1}$) concentrations to compute $EC_{50}$ values. Nevertheless, MCCP-P and MCCP appeared to be less toxic than all of the pesticides previously tested with the exception of being similarly toxic to the degradation product of glyphosate (AMPA; Mottier et al., 2013). Compared to the other compound families (metals, PAHs, by-products of adjuvants including nonylphenol, fungal peptides), the three herbicide molecules tested in the current study appeared to be weakly toxic to embryo-larval development in *C. gigas* (Table 3).

Few data concern the modes of action of contaminants on embryo-larval stages. Poirier and her collaborators (2007) have reported that fungal peptides (peptaibols detected in marine sediments and mussels) exhibit a broad spectrum of bioactivity, and larval abnormalities could be the result of a particular chemical interference of peptaibols with embryolarval development in relation to their membrane interaction properties. The chloroacetanilide herbicide metalochlor might affect the protein synthesis of oyster embryos and cause developmental defects and DNA damage (Mai et al., 2012). A significant positive correlation was demonstrated between embryotoxicity and genotoxicity

**Table 3**
Ecotoxicological data obtained from embryotoxicity test in *Crassostrea gigas*.

| Chemical | Chemical type | Ecotoxicological parameter | Values | References |
|---|---|---|---|---|
| Arsenic | Metal | $EC_{50}$ 48 h | 326 $\mu g\,L^{-1}$ | Martin et al. (1981) |
| Chrome(VI) | Metal | $EC_{50}$ 48 h | 4538 $\mu g\,L^{-1}$ | |
| Lead | Metal | $EC_{50}$ 48 h | 758 $\mu g\,L^{-1}$ | |
| Nickel | Metal | $EC_{50}$ 48 h | 349 $\mu g\,L^{-1}$ | |
| Selenium | Metal | $EC_{50}$ 48 h | Not reached (>10,000 $\mu g\,L^{-1}$) | |
| Zinc | Metal | $EC_{50}$ 48 h | 119 $\mu g\,L^{-1}$ | |
| Atrazine-simazine | Triazine herbicides | NOEC 24 h | 1000 $\mu g\,L^{-1}$ | Robert et al. (1986) |
| Mercury chloride | Metal | $EC_{50}$ 24 h | 12.3 $\mu g\,L^{-1}$ | His et al. (1999) |
| Mercaptodimethur | Carbamate pesticide | $EC_{50}$ 24 h | Not reached (>200 $\mu g\,L^{-1}$) | |
| Glyphosate | Phosphonoglycine herbicide | $EC_{50}$ 24 h | Not reached (>200 $\mu g\,L^{-1}$) | |
| Dinoterbe | Dinitrophenol herbicide | $EC_{50}$ 24 h | 72.2 $\mu g\,L^{-1}$ | |
| 4-Nonylphenol | Alkylphenol | NOEC 48 h | 10 $\mu g\,L^{-1}$ | Nice et al. (2000) |
| Benzo[a]pyrene | PAH | $EC_{50}$ 48 h | 2.5 $\mu g\,L^{-1} < CE_{50} < 25\ \mu g\,L^{-1}$ | Lyons et al. (2002) |
| Pyrene | PAH | $EC_{50}$ 48 h | ≈100 $\mu g\,L^{-1}$ | |
| Alamethicins | Fungal peptides | $EC_{50}$ 22 h | 31 nM | Poirier et al. (2007) |
| Long-chain peptaibols | Fungal peptides | $EC_{50}$ 22 h | 10 nM | |
| Short-sequence peptaibols | Fungal peptides | $EC_{50}$ 22 h | 64 nM | |
| Benzo[a]pyrene | PAH | LOEC 20 h | 0.05 $\mu g\,L^{-1}$ | Wessel et al. (2007) |
| 17α-Ethinylestradiol | Synthetic hormone | $EC_{50}$ 20 h | Not reached (>0.5 $\mu g\,L^{-1}$) | |
| Endosulfan | Organochlorine pesticide | NOEC 20 h | 61.04 $\mu g\,L^{-1}$ | |
| Diuron | Phenylurea herbicide | $EC_{50}$ 24 h | <0.05 $\mu g\,L^{-1}$ | Akcha et al. (2012) |
| Glyphosate | Phosphonoglycine herbicide | NOEC 24 h | 2.5 $\mu g\,L^{-1}$ | |
| Roundup Express® | Herbicide commercial formulation | $EC_{50}$ 24 h | not reached (>5 $\mu g\,L^{-1}$) | |
| Opus® | Epoxinocazole | $EC_{50}$ 24 h | 32.2 $\mu g\,L^{-1}$ | Amara (2012) |
| Nonyphenol | Alkylphenol | $EC_{50}$ 24 h | 20.9 $\mu g\,L^{-1}$ | |
| Irgarol | Triazine herbicide | NOEC 24 h | 0.001 $\mu g\,L^{-1}$ | Mai et al. (2012) |
| Metolachlor | Chloroacetamide herbicide | NOEC 24 h | 0.001 $\mu g\,L^{-1}$ | |
| Cadmium chloride | Metal | $EC_{50}$ 24 h | 212.3 $\mu g\,L^{-1}$ | |
| Arsenic (III) | Metal | $EC_{50}$ 24 h | 1370 $\mu g\,L^{-1}$ | Mamindy-Pajany et al. (2013) |
| Arsenic (V) | Metal | $EC_{50}$ 24 h | 920 $\mu g\,L^{-1}$ | |
| Glyphosate | Phosphonoglycine herbicide | $EC_{50}$ 48 h | 28,315 $\mu g\,L^{-1}$ | Mottier et al. (2013) |
| Amino methyl phosphonique acid | Biodegradation compound | $EC_{50}$ 48 h | 40,617 $\mu g\,L^{-1}$ | |
| Roundup express® | Herbicide commercial formulation | $EC_{50}$ 48 h | 1133 $\mu g\,L^{-1}$ | |
| Roundup Allées et Terasses® | Herbicide commercial formulation | $EC_{50}$ 48 h | 1672 $\mu g\,L^{-1}$ | |

(assessed by the comet assay) in *C. gigas*, particularly when exposed to benzo[a]pyrene (Wessel et al., 2007). These authors (2007) highlighted the ability of the oyster to biotransform the tested xenobiotics from early life stages and the bioactivation resulting in the induction of an oxidative stress involved in the measured oxidative DNA damages.

### 4.4. Comparison between different endpoints

When the $EC_{50}$ values of both endpoints are compared, it appears that embryos and D-shaped larvae are more sensitive than pediveliger larvae to MCPP and MCPP-P (present study) and to glyphosate-based herbicides (Mottier et al., 2013). The sensitivity of the very early stages can be linked to the very important morphological and anatomical changes occurring during embryo-larval development (Gilbert, 2003); many changes also occur during metamorphosis in *C. gigas* (Baker and Mann, 1994), but most likely not to the same extent. Quite surprisingly, the mecoprop degradation product (2-MCP) appeared to be more toxic to pediveliger larvae ($EC_{50}$ of 7199 $\mu g\,L^{-1}$) than to embryos and D-shaped larvae ($EC_{50}$ of 10,810 $\mu g\,L^{-1}$). In contrast with the embryotoxicity test, the metamorphosis assay has rarely been employed as an ecotoxicological endpoint in aquatic organisms. His and his collaborators (1997) used this endpoint applied to *C. gigas* to assess the toxicity of sediments polluted by HAPs, and they concluded that metamorphosis failure was a valid bioindicator of general toxicity. Moreover, metamorphosis is a critical period, especially crucial in terms of the recruitment and sustainability of wild stocks. In this context, metamorphosis of the Pacific oyster can be recommended in marine ecotoxicology; moreover, this endpoint can sometimes be more sensitive than the embryotoxicity test, which is especially known for its high sensitivity.

Comparisons of the current results with different endpoints recorded in ecotoxicological model species are difficult, but *C. gigas* early stages appeared to be relatively sensitive, especially for MCPP and MCPP-P. In fact, the results for oyster embryo-larval stages showed lower $EC_{50}$ values than those indicated for fish lethality, for a *D. magna* immobilisation test (PPDB, 2013) or for lethality to the copepod *N. spinipes* (Linden et al., 1979). However, both the $EC_{10}$ and $EC_{50}$ values computed in the current study are above the Predictive No Effect Concentrations (PNEC) proposed for saltwater: 18 $\mu g\,L^{-1}$ (long-term) and 187 $\mu g\,L^{-1}$ (short-term) (Whitehouse, 2010). Finally, the three tested molecules could significantly affect embryo-larval development and metamorphosis but at concentrations far higher than those recorded in aquatic environments (maximum values of a few tens of $\mu g\ L^{-1}$; Fletcher et al., 1995; Wittmer et al., 2010).

## 5. Conclusion

Mecoprop formulations (MCPP and MCPP-P) and their degradation compound (2-MCP) were found to be “slightly toxic” or “harmful” to oyster embryo-larval development according to the US EPA Classification and the European Toxicity Classification,

respectively. Considering these classifications, the parent molecules could be considered "practically nontoxic" in terms of metamorphosis mechanisms, whereas 2-MCP was classified as "moderately toxic" (or "toxic"). Nevertheless, the $EC_{50}$ values allowing these classifications were much higher than the concentrations predicted or measured in coastal environments.

Furthermore, the two enantiomers acted differently on non-target organisms (MCPP more toxic to embryo-larval development) compared to target organisms (MCPP-P exhibiting the herbicidal activity). The herbicides' toxicity appeared to depend not only on the molecule but also on the chirality of this molecule. For both endpoints, 2-MCP appeared to be more toxic than MCPP and MCPP-P, and a quite surprising result was that the by-product was more toxic to pediveliger larvae (21 days old) compared to D-shaped larvae (36 h old). In addition to active compounds and commercial formulations, it is thus important that regulations consider degradation products, which can be more harmful to the environment. The mode of action of MCPP on *C. gigas* D-shaped larvae remains unknown, but in accordance with the scarce literature, this study indicated a potential effect of this molecule on shell development, with percentages of abnormal shells and a severity of shell deformities that are higher than those recorded with other molecules.

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