*Review*

# The Effects of Glyphosate and Its Commercial Formulations to Marine Invertebrates: A Review

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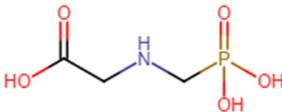
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**Abstract:** Glyphosate is the active ingredient of numerous commercial formulations of herbicides applied in different sectors, from agriculture to aquaculture. Due to its widespread use around the world, relatively high concentrations of glyphosate have been detected in soil and aquatic environments. The presence of glyphosate in aquatic ecosystems has aroused the attention of researchers because of its potential negative effects on living organisms, both animals and plants. In this context, this review intends to summarize results of studies aimed at evaluating the effects of glyphosate (both as active ingredient and component of commercial formulations) on marine invertebrates. Generally, data obtained in acute toxicity tests indicate that glyphosate and its commercial formulations are lethal at high concentrations (not environmentally realistic), whereas results of long-lasting experiments indicate that glyphosate can markedly affect biological responses of marine invertebrates. Consequently, more efforts should be addressed at evaluating chronic or sub-chronic effects of such substances to marine invertebrate species.

**Keywords:** glyphosate; herbicide; aquatic toxicology; marine invertebrates

## 1. Introduction

Glyphosate, also known as N-(phosphonomethyl) glycine [CAS registry number 1071-83-6], is a broad-spectrum organophosphate herbicide with a non-selective, post emergence, and systemic activity (Table 1). It is absorbed by leaves and translocated through the phloem in all plant districts [1], but plants can also uptake the herbicide from roots and translocate it through the xylem [2]. Glyphosate affects the shikimate pathways inhibiting the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), an enzyme which catalyzes the penultimate step in the shikimate pathway [3,4]. The inhibition lead to a decrease of the three essential aminoacids, tyrosine, phenylalanine, and tryptophan, as well as a possible decrease of second metabolites such as flavonoids, lignin, and phytoalexins [5,6]. EPSPS is an enzyme present in all plants, both herbaceous and arboreal, in fungi, algae, and some microorganisms such as Apicomplexa on which glyphosate can also act [7–10]. In addition, glyphosate, which has chelating properties, may affect the plant bioavailability of several elements, both reducing their uptake or mobilizing it [11–13], but these aspects are still debated [5,6]. Also, glyphosate can reduce the resistance of plants to pathogens [6,14]. Besides glyphosate mainly being used in agriculture with about 1.2 million $km^2$ of crop land treated annually alone in the United States (U.S.) [15], it is also applied in non-agricultural sectors such as forestry, urban, and resident weed control practices, in the control of aquatic weeds and along railroads [15–18].

Table 1. Chemical and physical properties of glyphosate.

| IUPAC Name | N-(phosphonomethyl) Glycine |
|---|---|
| CAS Number | 1071-83-6 |
| Molecular Formula | $C_3H_8NO_5P$ |
| Chemical Structure |  |
| Molecular Weight | 169.07 g/mol |
| Physical state and Color | White crystalline powder |
| Melting Point | 189.5–230 °C |
| Density at 20 °C | 1.705 |
| Water Solubility at 25 °C | 1.2 g/100 mL |
| Octanol-water Coeff. (log Kow) | −3.40 to −1.0 |
| Vapor Pressure at 25 °C | $1.31 \times 10^{-2}$ mPa |

Glyphosate's "history" starts in 1950 when the swiss chemist Henri Martin discovered it. However, the herbicide reached the market only in 1974 [19]. In almost 50 years, several glyphosate-based herbicides (GBHs) have been developed (e.g., Accord, Aquamaster, Glyfos, Roundup, Rodeo, Touchdown) and over than 750 GBHs are registered in the U.S. and around 500 in Australia [20,21]. GBHs contain a wide spectrum of glyphosate salts, such as isopropylamine, diammonium, monoammonium, potassium, trimethylsulfonium, and sesquisodium [22]. Adjuvants, usually surfactants, are also present to enhance the glyphosate absorption trough the cuticle of the leaves, as commonly performed for several herbicides [23]. Adjuvants commonly present in GBHs include polyethoxylated tallow amine (POEA), quaternary ammonium compounds, polyoxyethylene alkyl ether phosphates, and alkyl polyglucoside, but usually they are listed as generic surfactants by the manufactures [24,25].

In terms of production, glyphosate is the most worldwide selling herbicide [26] and GBHs are used in more than 130 countries on more than 100 crops [27]. In the U.S., for example, glyphosate is the most commonly used pesticide in agriculture since 2001 and the second most used active ingredient in the non-agricultural sectors since 2005 [28]. In the U.S. agricultural sector its usage increased 300-fold from 1974 to 2014, particularly after the introduction of genetically herbicide resistant crops in 1990s with over 113 thousand tons used in 2014 [29]. As for Asian region, the main users of glyphosate are China (which is also the main glyphosate producer) and India [30]. Interestingly, glyphosate import increased by 177% in South Africa [31]. Globally, the estimated use of glyphosate (both agricultural and non-agricultural) was 56.296 tons in 1994 and has risen to 825.804 tons in 2014 [29] with an expected increase to 740–920 thousand tons in 2025 [32].

## 1.1. Environmental Fate of Glyphosate

The half-life of glyphosate in soil is affected by mineral and organic composition, climate conditions, and microbial activity [33–36]. In soil, the half-life values are in the order of 1.7–197 days [37], 30–174 days [38], 143.3 days [39], and 16.9–151 days [40], but in some case glyphosate remain detectable even after several months [41]. As for freshwater ecosystems, Annett et al. [42] reported a half-life of 7–142 days. Other authors recorded half-life values for surface waters between 1.5–16 days [43–46], while Mallat and Barceló [47] observed a half-life of 60–770 hours for groundwater. Surprisingly, Pizzarro et al. [48] report a range of 31.5-33.5 weeks. In seawater, the glyphosate half-life was estimated to be 47 ± 7 days at 25 °C under light condition, 267 ± 21 days at the same temperature but in the dark and, 315 ± 29 days in the dark at 31 °C [49].

Environmental glyphosate degradation is mainly mediated by microbial activity [50], while the compound is more resistant to photolysis and chemical decomposition [38,47]. Moreover, an abiotic degradation pathway of glyphosate due to the manganese mineral birnessite, commonly present

in soil, has also been proposed [51,52]. Bacteria and fungi degrade glyphosate through two main biochemical pathways [36,53,54]: C-P lyase pathway and AMPA pathway [6,38,55], allowing them to use the herbicide as a source of P, N and C [36,56] (Figure 1).
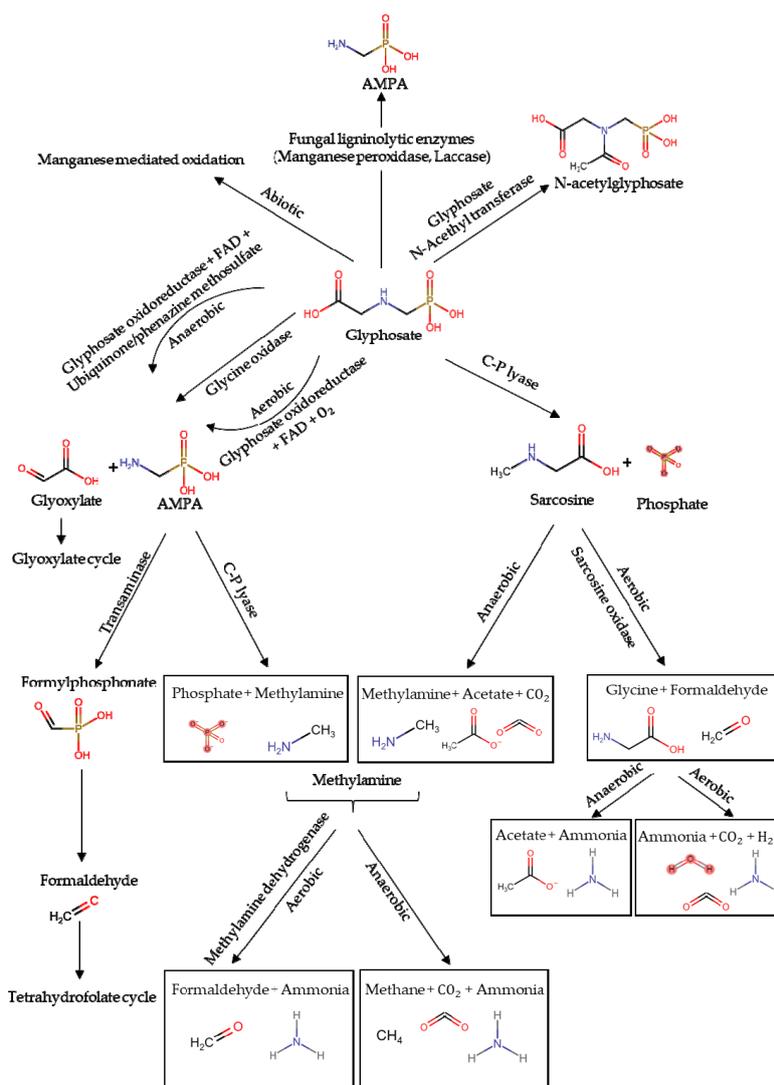


**Figure 1.** Main degradation pathways of glyphosate. Oxygen, water molecules and hydrogen atoms were omitted to facilitate the visualization.

In the first degradation pathway glyphosate is oxidized in aerobic conditions by glyphosate oxidoreductase using FAD as cofactor and $O_2$, or alternatively FAD and ubiquinone or phenazine methosulfate as electron acceptors in anaerobic conditions, making glyoxylate and its main metabolite aminomethylphosphonic acid (AMPA) [36]. Alternatively, also glycine oxidase degrades the herbicide to glyoxylate and AMPA [53]. Then, glyoxylate is used in the cellular glyoxylate cycle while AMPA can be excreted in the soil or even further biodegraded by C-P lyase to inorganic phosphate and methylamine. In aerobic conditions, methylamine is converted by methylamine dehydrogenase in ammonia and formaldehyde which is used in the tetrahydrofolate cycle. Methylamine can also be degraded anaerobically in ammonia, methane, and $CO_2$ [38,54]. A different AMPA degradation pathway has been observed in which the compound is converted in formylphosphonate by a transaminase enzyme and then to formaldehyde [56,57]. In the second pathway the herbicide is degraded in sarcosine and inorganic phosphate by C-P lyase pathway trough several chemical reactions [58]. Sarcosine is then degraded by sarcosine oxidase in formaldehyde (see above for its degradation) and glycine

which is commonly used in cellular cycles [57]. In aerobic conditions, glycine is then degraded in ammonia, water, and $CO_2$, while in anerobic soil it is converted in acetate and ammonia. Alternately, sarcosine is converted in methylamine, acetate, and $CO_2$ [54]. In a third pathway, glyphosate can be converted by glyphosate N-acetyltransferase in N-acetylglyphosate, but its further biodegradation remains unclear [53,59]. Finally, it has also been proposed that two fungi ligninolytic enzymes, laccase and manganese peroxidase, are able to degrade glyphosate in AMPA, but biochemical details remain unclear [60].

### 1.2. Occurrence of Glyphosate in Aquatic Environments

Despite the high affinity of glyphosate for soil particles and its consequent low mobility [61,62], it has been detected in a wide spectrum of water bodies (Table 2). Indeed, glyphosate can reach surface waters through either runoff and soil leaching or more rarely through a direct application into water (e.g., to control aquatic weeds) [15,36]. In groundwater, herbicide can occur due to karst phenomena, as reported in intensive agricultural areas, but it rarely reaches high levels in groundwater because it remains bind to soil particles [36]. Yang et al. [63], for example, reported that up to 14% of applied glyphosate can reach the water bodies due to runoff, while the European Food Safety Authority (EFSA) [64] has calculated a dissipation half-life ($DT_{50}$) of 13.8–301 days in river.

Concentrations of glyphosate in water can vary along seasons and depends on rainfalls intensities [65–68] making it detectable not only in soil, but also in Wastewater Treatment Plants (WWTPs), agricultural areas surface water, groundwater, rivers and seawater [66–116]. According to Kemp et al. [73], who calculated that up to 2% of pesticide reach the marine environment, glyphosate has been observed in estuarine waters and seawaters [74–76]. In addition, glyphosate has also been detected in precipitations and phytotelmic water [69,72,77]. Surprisingly, despite its low vapor pressure, glyphosate has also been detected in atmosphere [78]. Overall, the environment concentrations are usually in order of few μg/L (see Table 2), much less than the worst scenario (up to 5.4 mg/L) [79]. However, high concentration values are detected in agricultural water bodies even up to hundreds of μg/L [66,80,81].

**Table 2.** Occurrence of glyphosate in water ecosystems. Data are reported as <LOD (limit of detection) up to the highest measured concentration.

| Glyphosate Concentrations | Water Environment, Country | Ref. |
| --- | --- | --- |
| 0.10 to 0.70 mg/L | Surface waters, Argentina | [66] |
| <LOD-427 μg/L | Surface waters, USA | [69] |
| <LOD-430 μg/L | Surface waters, USA | [80] |
| <LOD-1600 μg/L | Surface waters, Argentina | [81] |
| <LOD-4.52 μg/L (2.11 * μg/L) | Surface waters, Argentina | [82] |
| <LOD-36.71 μg/L (3.02 * μg/L) | Surface waters, Mexico | [83] |
| <LOD-27.8 μg/L (1.68 ** μg/L) | Surface waters, USA | [67] |
| <0.1–427 μg/L | Surface waters, USA | [72] |
| <LOD-2.1 μg/L | Surface waters, Italy | [84] |
| <LOD-8.2 μg/L (0.4 * μg/L) | Surface waters, Argentina | [70] |
| LOD-2.9 μg/L (0.78 * μg/L) | Surface waters, Argentina | [85] |
| <LOD-4970 ng/L | Surface waters, Switzerland | [86] |
| <LOD-145 ng/L | Surface waters, Switzerland | [87] |
| 15–390 ng/L | Surface waters, Switzerland | [71] |

**Table 2.** *Cont.*

| Glyphosate Concentrations | Water Environment, Country | Ref. |
|---|---|---|
| <LOD-90 µg/L | Surface waters, Poland | [88] |
| <LOD-0.08 µg/L | Surface waters, USA | [89] |
| <LOD-2.1 µg/L (0.11 ** µg/L) | Surface waters, Switzerland | [90] |
| 1.258–1.550 mg/L | Surface waters, Brazil | [91] |
| < LOD-1.93 µg/L | Surface waters, Austria | [92] |
| <2–3000 ng/L (109 * ng/L; 26.9 ** ng/L) | Surface waters, Canada | [93] |
| 10 mg/L | Surface waters, China | [94] |
| <LOD-7.6 µg/L | Surface water, Argentina | [95] |
| <LOD-18 µg/L | Surface waters, Canada | [96] |
| <LOD-0.7 µg/L (0.6 * µg/L) | Surface water, Argentina | [97] |
| <LOD-12 µg/L | Surface waters, Canada | [98] |
| <LOD-0.08 µg/L | Surface waters, Italy | [99] |
| <LOD-11.8 µg/L (158.6 * ng/L; 19.8 ** ng/L) | Surface waters, Canada | [100] |
| <LOD-0.041 mg/L | Surface waters, Brazil | [101] |
| <LOD-8.1 µg/L (0.05 ** µg/L) | Surface waters, USA | [102] |
| <LOD-0.68 ng/mL | Surface water, Hungary | [103] |
| <LOD-40.8 µg/L | Surface water, Canada | [104] |
| <LOD-125 µg/L | Surface waters, Argentina | [105] |
| <LOD-455 ng/L | Surface water, Canada | [106] |
| <LOD-14.2 µg/L | Surface waters, Australia | [68] |
| <LOD-2.2 µg/L | Surface waters, USA | [107] |
| <LOD-8.7 µg/L | Surface waters, USA | [108] |
| <LOD-90 µg/L | Surface waters, France | [65] |
| <LOD-31 µg/L | Surface waters, Denmark | [109] |
| <LOD-59.9 µg/L | Surface waters, USA | [110] |
| <LOD-165 µg/L | Surface waters, France | [111] |
| <LOD-1.3 µg/L | Surface waters, Belgium | [112] |
| <LOD-0.46 µg/L | Surface waters, Finland | [112] |
| <LOD-50 µg/L | Surface waters, France | [112] |
| <LOD-4.7 µg/L | Surface waters, Germany | [112] |
| <LOD-1.8 µg/L | Surface waters, Ireland | [112] |
| <LOD-11 µg/L | Surface waters, Italy | [112] |
| <LOD-0.93 µg/L | Surface waters, Norway | [112] |
| <LOD-3.6 µg/L | Surface waters, Slovakia | [112] |
| <LOD-15.3 µg/L | Surface waters, Spain | [112] |
| <LOD-13 µg/L | Surface waters, Sweden | [112] |
| <LOD-8.8 µg/L | Surface waters, UK | [112] |
| <LOD-3.6 µg/L | Surface waters, Austria | [112] |
| <LOD-8.7 µg/L | Groundwaters, Denmark | [112] |

**Table 2.** *Cont.*

| Glyphosate Concentrations | Water Environment, Country | Ref. |
| --- | --- | --- |
| <LOD-24 µg/L | Groundwaters, France | [112] |
| <LOD-1.2 µg/L | Groundwaters, Italy | [112] |
| <LOD-1.7 µg/L | Groundwaters, Sweden | [112] |
| <LOD-0.21 µg/L | Groundwaters, Switzerland | [112] |
| <LOD-4.7 µg/L | Groundwaters, Netherlands | [112] |
| <LOD-0.47 µg/L | Groundwaters, UK | [112] |
| <LOD-6.8 µg/L | Groundwaters, France | [111] |
| <LOD-0.67 µg/L | Groundwaters, Denmark | [109] |
| <LOD-0.98 ng/mL | Groundwaters, Hungary | [103] |
| <LOD-0.011 µg/L | Groundwaters, Italy | [99] |
| <LOD-2.56 µg/L (202 * ng/L) | Groundwaters, Spain | [113] |
| <LOD-0.025 µg/L | Groundwaters, Switzerland | [90] |
| <LOD-42 ng/L | Groundwater, Canada | [114] |
| <LOD-8.5 µg/L (0.4 * µg/L) | Groundwaters, Argentina | [70] |
| 0.44–1.41 µg/L | Groundwaters, Mexico | [115] |
| <0.1–4.7 µg/L | Groundwaters, USA | [72] |
| <LOD-663 ng/L | Groundwaters, Canada | [116] |
| <LOD-1690 ng/L (up to 665 * ng/L) | Sea water, Baltic Sea estuaries | [76] |
| 13–1377 µg/L | Sea waters, Western Pacific | [75] |
| <LOD-1.2 µg/L | Sea waters, French Atlantic coast | [74] |
| <0.1–2.5 µg/L (0.1–0.2 ** µg/L) | Precipitations, USA | [78] |
| 0.3–1.1 µg/L | Precipitations, USA | [72] |
| <LOD-135 ng/L | Precipitation, Canada Ontario | [116] |
| 0.2210–5 µg/L | Phytotelmic water, Belize | [77] |

When available, mean * and median ** concentrations are reported in parentheses.

## 2. The Effects of Glyphosate to Marine Invertebrates

To our knowledge, most of the studies that have been performed to evaluate the toxic effects of glyphosate to non-target marine invertebrates are mainly related to mollusks (Table 3). However, in this review an attempt was also made to summarize the results obtained with other invertebrates.

### 2.1. Mollusks

Different species of marine mollusks have been used as model organisms to assess the effects of glyphosate and its commercial formulations on different levels of animal biological organization. For example, gametes and embryos from oysters (*Crassostrea gigas*) were used to assess the impact of glyphosate (as active ingredient) and Roundup® (commercial formulation) on population dynamics [117]. Three independent embryo-larval bioassays were conducted, but only one demonstrated that glyphosate (at 2.5 and 5 µg/L) can exert embryotoxic effects in term of increases of abnormal D-larvae percentage. Cumulative results of the three assays revealed a significant increase in the percentage of abnormal D-larvae only at 5 µg/L, whereas Roundup® did not affect significantly oyster embryos (as cumulative data from three independent bioassays). Neither glyphosate nor Roundup® exerted genotoxic effects on oyster spermatozoa.

**Table 3.** Effects of glyphosate (as active ingredient) and its commercial formulations in mollusks. *Abbreviations*: **AChE** (acetylcholinesterase); **ALP** (alkali labile phosphate); **GST** (glutathione S-transferases); **HL** (hemocyte lysate); **REX**: Roundup Express®; **THC** (total hemocyte count); **Vtg** (vitellogenin).

| Compound Tested | Concentrations (Exposure Type) | Species | Effects | Ref. |
|---|---|---|---|---|
| Glyphosate (active ingredient) Roundup (commercial formulation) | 0.5, 1, 1.5, 2.5, 5 µg/L (*in vitro* exposure) | *Crassostrea gigas* (gametes and embryos) | increases in the percentage of abnormal D-larvae no genotoxic effects on oyster spermatozoa | [117] |
| Glyphosate (acid, 97% purity) | 0.1, 1, 100 µg/L (*in vivo* exposure) | *Crassostrea gigas* (juveniles) | no mortality no growth no histological alterations moderate alterations of enzyme activities moderate alterations in gene expression | [118] |
| Roundup Ready-To-Use Plus® | 0.25, 1, 4, 16 mg/L (*in vitro* exposure) | *Crassostrea virginica* (sperm) | no significant alterations in mitochondrial membrane potential in the sperm | [119] |
| Glyphosate (active ingredient) | 2 µg/L (*in vivo* exposure) | *Crassostrea gigas* (adults) | gills: decreases in expression of GSTs digestive gland: increases in expression of GSTs | [120] |
| Glyphosate (active ingredient) | 2 µg/L (*in vivo* exposure) | *Crassostrea gigas* (adults) | differentially regulated gene expression in gills and digestive gland | [121] |
| Glyphosate (active ingredient) | 10, 100, 1000 µg/L (*in vivo* exposure) | *Mytilus galloprovincialis* (adults) | effects on the transcriptional regulation of genes involved in important cell functions | [122] |
| Glyphosate (active ingredient) | 10, 100, 1000 µg/L (*in vivo* exposure) | *Mytilus galloprovincialis* (adults) | alterations in hemocyte parameters no marked alteration in antioxidant enzyme activity in gills and digestive gland alterations in gill AChE activity | [123] |
| Glyphosate (active ingredient) | 10, 100, 1000 µg/L (*in vivo* exposure) | *Ruditapes philippinarum* (adults) | reductions in THC increases in hemocyte volume and diameter increases in hemocyte proliferation increase in HL acid phosphatase activity | [124] |
| Glyphosate (pure) | from 0.075 to 15 mM (*in vitro* exposure) | *Perna perna* (tissue from juveniles) | significant inhibition of cholinesterase activity in gills and muscle | [125] |
| Roundup Express® (REX) | 0.1, 1, 100 µg/L (*in vivo* exposure) | *Crassostrea gigas* (juveniles) | no mortality no effects on condition index delay in gametogenesis decreases in shell length slight reduction in whole weight moderate alterations of digestive gland enzyme activities | [126] |
| Glyphosate (active ingredient) REX (commercial formulation) | from 0.1 to 100,000 µg/L (*in vitro* exposure) | *Haliotis tuberculata* (hemocytes) | cell viability: significant reduction due to REX, but not to glyphosate phagocytosis: significant reduction due to REX, but not to glyphosate lysosomal stability: significant effects of low concentrations of glyphosate and high levels of REX | [127] |
| Roundup® 3plus | 0.2, 1 g/L (*in vivo* exposure) | *Ruditapes decussatus* (adults) | alteration of energy metabolism and metabolic biomarkers | [128] |
| Roundup® Power 2.0 | 100, 1000 µg/L (*in vivo* exposure) | *Mytilus galloprovincialis* (adults) | no significant alterations in *vtg* gene expression in female gonads reduction in ALP levels if female gonads at 21 days reduction in ALP levels if male gonads at 7 days and increases at 21 days | [129] |

In the same oyster species (*C. gigas*), the effects of long-term exposure (56 days) to glyphosate (0.1, 1, and 100 µg/L) were evaluated in juvenile oysters by means of different endpoints measured at both molecular and individual levels [118]. At the individual level, biomarkers (e.g., growth, condition

index, sexual maturity, and tissue alterations) revealed a moderate effect of glyphosate. Neither mortality nor growth were detected during the study. Histological analyses revealed the absence of significant tissue alterations (atrophies of the wall of the digestive tubules and destructuration of the connective tissue), whereas a significant increase in hemocyte infiltration was detected between oysters at T0 and those at 56 days. Moderate effects of glyphosate were also recorded on oyster enzymatic activities, lipid peroxidation and expression of reference genes under the experimental conditions tested. In that study, the authors hypothesized that the low toxicity of glyphosate could be due to its chemical features, such as high-water solubility and moderate bioaccumulation potential [118].

The effects of different concentrations of the glyphosate-based herbicide Roundup Ready-To-Use Plus® (0.25, 1, 4, and 16 mg/L glyphosate) were assessed in the oyster *Crassostrea virginica* by means of sperm viability biomarkers [119]. Exposure for 20 min of *C. virginica* sperm to the herbicide did not alter significantly mitochondrial membrane potential in the sperm, as measured with MitoTracker Red CMXRos® [119]. Although cellular respiration did not decrease in sperm samples from the two bivalve species after Roundup exposure, the increase (not statistically significant for *C. virginica*) in the percentage of samples emitting high fluorescence intensity when exposed to 16 mg/L Roundup was probably due to plasma membrane damage, which in turn led to increased intracellular pH in the cytoplasm and polarization in the mitochondria [119].

A semi-quantitative multiplex RT-PCR (real-time polymerase chain reaction) method was used to evaluate mRNA expression of four different classes of glutathione S-transferases (GST)— *pi, sigma, mu*, and *omega* - in the Pacific oyster *C. gigas* exposed for 4 weeks to glyphosate (2 µg/L) [120]. Despite *mu* class of GST was not expressed in both gills and digestive gland from oysters, the expression of the remaining GSTs was markedly inhibited in gills, mainly after 30 days of exposure, while the expression generally increased in digestive gland [120]. In that study, the authors suggested that measurement of mRNA expression of *pi* and *sigma* class GST can be a useful biomarker of pesticide exposure in oysters. In the same oyster species, a suppression subtractive hybridization (SSH) method was used to reveal up and down-regulated genes following 30 days of exposure to glyphosate [121]. Identified genes from the SSH libraries resulted expressed differentially in gills and digestive gland from glyphosate-exposed oysters, compared to the control [121].

In the mussel *Mytilus galloprovincialis*, digestive gland transcriptional profiles were investigated through RNA-seq following exposure for 21 days to 10, 100, and 1000 µg/L [122]. A total of 111, 124, and 211 differentially regulated transcripts was found in mussels exposed to 10, 100, and 1000 µg/L, respectively. Five genes were shown to be differentially expressed at all glyphosate concentrations, including SERP2 (with a role in the protection of unfolded target proteins against degradation), GIMAP5 (an antiapoptotic protein) and MTMR14 (with a role in macroautophagy). At the functional level, that study revealed that several important biological functions, such as cell signaling, energy metabolism, and $Ca^{2+}$ homeostasis were compromised, even at the lowest concentrations tested [122]. In the parallel study, we demonstrated that exposure for 7, 14, and 21 days to glyphosate (10, 100, and 1000 µg/L) affected significantly hemocyte parameters, such as total hemocyte count (THC, decreased at 7 and 14 days), hemocyte volume (increased at 7 and 21 days) hemolymph pH (increased after 14 days of exposure), hemocyte lysate lysozyme (reduced at 7, 14, and 21 days), and acid phosphatase (decreased at 7 days, increased at 14, and reduced again at 21 days) activities and cell membrane stability (lactate dehydrogenase activity increased in cell-free hemolymph of mussels exposed for 7 and 14 days) [123]. Conversely, exposure to glyphosate did not markedly alter antioxidant enzyme activities in both gills and digestive gland [123]. Overall, that study demonstrated that glyphosate affected mostly hemocyte functionality. Hemocyte parameters were also measured in the clam *Ruditapes philippinarum* to confirm the effects of glyphosate at the cellular level [124]. In that study, exposure for 7 days to glyphosate (10, 100, and 1000 µg/L) was shown to significantly reduce THC, whereas hemocyte diameter and volume increased significantly in treated clams. In addition, hemocyte proliferation and hemocyte lysate acid phosphatase activity increased significantly in glyphosate-exposed clams. That study demonstrated

further that hemocytes (circulating cells that play a key role in immune defenses) are a target for glyphosate action in marine bivalves.

Although glyphosate is not considered an acetylcholinesterase (AChE) inhibitor, some in vitro and in vivo studies demonstrated that such compound may affect neurotransmission in mollusks. For example, pure glyphosate concentrations (from 0.075 to 15 mM) were shown to inhibit cholinesterase activity in vitro in a concentration-dependent manner in gills and muscle from the marine brown *Perna perna*, inhibition of enzyme activity reaching more than 50% even at the lowest concentrations tested (0.75–1 mM) [125]. In our in vivo study, exposure for 7, 14, and 21 days of *M. galloprovincialis* to glyphosate was shown to reduce significant AChE activity in gills, even if a non-linear response of enzyme activity was recorded. Indeed, AChE activity decreased at 7 days, increased at 14 days, and decreased after 21 days of exposure [123]. The increase in AChE activity in mussels exposed for 14 days was probably a response of mussels to face the decrease at 7 days, whereas the pronounced inhibition of AChE at 21 days indicated inability of mussels to cope with the herbicide.

At the organism level, the effects of the glyphosate-based herbicide Roundup Express® (REX) containing adjuvants, such as polyethoxylated tallow amines (POEAs), have been evaluated in *C. gigas* juveniles [126]. Exposure for 35 days to three concentrations (0.1, 1, and 100 µg/L) of REX did not cause significant effects on mortality rates, histological biomarkers, and condition index (CI), whereas shell length decreased significantly after 35 days of exposure to 0.1 and 100 µg/L, as well as the whole weight of oysters treated for 14 days with the lowest concentration tested. Interestingly, a delay in gametogenesis was recorded in oysters exposed for 35 days to 0.1 and 1 µg/L. As for digestive gland biomarkers, that study revealed no significant effects of REX on total protein content, catalase (CAT), and GST activities and lipid peroxidation. The authors suggested that biomarkers measured at the individual level, such as shell growth and reproduction, are more responsive to chronic exposure to herbicides [126].

A comparative in vitro study on the effects of glyphosate (as active ingredient) and REX (commercial formulation) has been performed on hemocytes from the abalone *Haliotis tuberculata*, a marine gastropod species [127]. Several concentrations (from 0.1 to 100,000 µg/L) of both the substances were tested and the effects on cell viability, phagocytosis, and lysosomal membrane stability were evaluated following exposure for 72 h of hemocytes. Cell viability was not affected by glyphosate, even at the highest concentration tested (100,000 µg/L), whereas exposure to REX induced a significant decrease in hemocyte viability at 40,000 µg/L, reaching a dramatic reduction (about 7%) at 100,000 µg/L. Similarly, glyphosate did not affect phagocytic activity of hemocytes, whereas it decreased significantly in hemocyte treated with REX (from 10,000 µg/L). As for lysosomal membrane stability, that study demonstrated that the lowest concentration of glyphosate (0.1 µg/L)—but not the two highest (10,000 and 100,000 µg/L)—affected significantly lysosomal stability, when compared to controls. An opposite response was observed after REX exposure, with a significant decrease in neutral red retention in lysosomes of hemocytes treated with the highest concentrations tested (from 10,000 µg/L) [127]. Overall, the study demonstrated that the commercial formulation can be more harmful than the active ingredient for hemocytes, probably due to the presence of adjuvants, such as POEAs, used to increase glyphosate efficacy towards plants.

In another study, the effects of Roundup® 3plus on metabolic parameters of heart of the clam *Ruditapes decussatus* were evaluated by means of HRMAS NMR spectroscopy following exposure for 24 and 72 h to two doses of herbicide (0.2 and 1 g/L) [128]. As a result, exposure to the herbicide caused anaerobiosis and alterations of oxidative metabolism, as revealed by alteration in energy metabolism and metabolic biomarkers (e.g., alanine, succinate, acetate, and propionate). That study demonstrated usefulness of HRMAS NMR technique in investigating mechanisms of action of Roundup in marine bivalves.

Recently, potential estrogenic effects of a new glyphosate-based formulation, namely Roundup® Power 2.0, were evaluated in *M. galloprovincialis* [129]. Mussels were exposed for 7, 14, and 21 days to two concentrations of Roundup® Power 2.0, corresponding to 100 and 1000 µg/L of glyphosate.

In that study, no significant alterations in vitellogenin (Vtg) gene expression were recorded in female gonads, whereas a significant decrease in alkali labile phosphate (ALP, an indirect method for Vtg measurement) levels was observed in female gonads exposed for 21 days and in males exposed for 7 days. Interestingly, ALP levels increased significantly in gonads from males exposed for 21 days. Results demonstrated that the glyphosate-based formulation can affect reproduction-related parameters in mussels.

## 2.2. Other Marine Invertebrates

To the best of our knowledge, information concerning the effects of glyphosate or its commercial formulations to other marine invertebrates are limited to few taxonomic groups (Table 4).

As for crustacea, attention has been paid to the acute toxicity of glyphosate on different life stages of organisms. For example, the acute toxicity of two glyphosate-based formulations, namely Roundup® Original and Glyphosate AKB 480 (AKB), on early life stages of *Artemia salina* have been investigated [130]. Exposure of nauplii for 48h to 5, 10, 25, 50, and 100 mg/L (corresponding to 1.8, 3.6, 9, 18, and 36 mg/L of glyphosate) to both glyphosate-based herbicides induced significant increases in mortality rate, even if Roundup® was more toxic than AKB, 48-h $LC_{50}$ values being 14.19 mg of glyphosate acid equivalent/L and 37.53 mg/L of glyphosate acid equivalent, respectively. Conversely, 48-h $LC_{50}$ values of 1.77 mg/L, 49.3 mg/L and 35.3 mg/L were recorded after 48 h of exposure of *Acartia tonsa* to Roundup®, glyphosate acid and isopropylamine salt of glyphosate [131]. In the blue crab *Callinectes sapidus*, the acute toxicity of Roundup® Pro on crab megalopae and J1–J4 stage juveniles was investigated following exposure for 24h [132]. The resulting $LC_{50}$ values were 6279 µg/L for megalopae and 316,000 µg/L for juveniles, indicating that the post-larval stage is more sensitive than juveniles to Roundup® Pro. Overall, results of the three studies above [130–132] suggest that differences in $LC_{50}$ values can be due partially at least to different compounds tested and the species used along their development stage (nauplii of *A. salina*, adults of *A. Tonsa*, megalopae and juveniles of *C. sapidus*). Exposure for 90 days during the pre-reproductive period (winter) of females of the estuarine crab *Neohelice granulata* to glyphosate (0.02, 0.2, and 1 mg/L) caused a decrease in body weight at all the concentrations tested, whereas no alterations in gonadosomatic index and vitellogenic protein content of the ovary were recorded. However, a significantly increased percentage of reabsorbed vitellogenic oocytes was observed in crabs exposed to 1 mg/L, suggesting that glyphosate can affect both somatic and the ovarian growth in crabs [133]. In the same estuarine crab species, exposure of females for 90 days to Roundup Ultramax® (0.01 and 0.2 mg/L, acid equivalent) induced a significant increase in glycemia, while glycogen content in the muscle did not change significantly [134]. In addition, no significant effects of Roundup Ultramax® on gonadosomatic index were found, whereas a significantly higher percentage of reabsorbed vitellogenic oocyte and a significant decrease of vitellogenin content in the ovary were recorded at 0.2 mg/L. That study demonstrated that environmentally realistic concentrations of Roundup Ultramax® can affect significantly important reproduction-related parameters of crabs, such as ovary vitellogenin content and oocyte maturation [134]. Interestingly, the same group of researchers demonstrated that both glyphosate and its commercial formulation Roundup Ultramax® affected some important biological parameters in males of *N. granulate* [135]. Exposure for 30 days to 1 mg/L of both the compounds caused a significant decrease in weight and total proteins in muscle, and a significant increase in muscle glycogen content. Abnormal spermatophores—partially empty of spermatozoa—was observed following exposure to the two compounds. The authors concluded that 1-month exposure of crabs to glyphosate and its commercial formulation can affect the reproductive performance of male animals, by producing abnormal spermatophores and reducing sperm count [135].

**Table 4.** Effects of glyphosate (as active ingredient) and its commercial formulations in other marine invertebrates. *Abbreviations*: **AChE** (acetylcholinesterase); **LC50** (median lethal concentration); **ROS** (reactive oxygen species); **SOD** (superoxide dismutase); **Vtg** (vitellogenin).

| Compound Tested | Concentrations (Exposure Type) | Species Crustacea | Effects | Ref. |
|---|---|---|---|---|
| Roundup® Glyphosate AKB 480 | 5, 10, 25, 50, 100 mg/L, corresponding to 1.8, 3.6, 9, 18 and 36 mg/L of glyphosate (*in vivo* exposure) | *Artemia salina* (nauplii) | increased mortality rates, even if Roundup was more toxic than AKB | [130] |
| Glyphosate acid Isopropylamine salt of glyphosate Roundup® | 1 to 100 mg acid equivalent/L (*in vivo* exposure) | *Acartia tonsa* (adults) | $LC_{50}$ determination | [131] |
| Roundup® Pro | $10^3$ to $10^7$ µg/L (*in vivo* exposure) | *Callinectes sapidus* (megalopae and juvenile stages) | $LC_{50}$ determination | [132] |
| Glyphosate (active ingredient) | 0.02, 0.2, 1 mg/L (*in vivo* exposure) | *Neohelice granulata* (adult females) | decrease in body weight<br>no effects on gonadosomatic index<br>no effects on vitellogenic protein content of the ovary<br>increased percentage of reabsorbed vitellogenic oocytes | [133] |
| Roundup Ultramax® | 0.01 and 0.2 mg/L asacid equivalent (*in vivo* exposure) | *Neohelice granulata* (adult females) | increases in glycemia<br>no effects on muscle glycogen content<br>no effects on gonadosomatic index<br>increased percentage of reabsorbed vitellogenic oocytes<br>decrease in Vtg levels in the ovary | [134] |
| Glyphosate (as-active ingredient) Roundup Ultramax® | 1 mg/L (*in vivo* exposure) | *Neohelice granulata* (adult males) | reductions in weight<br>reductions in total protein levels in muscle<br>increase in glycogen content in muscle<br>increased percentage of abnormal spermatophores | [135] |
| **Echinoderms** | | | | |
| Roundup Ready-To-Use Plus® | 0.25, 1, 4, 16 mg/L (*in vitro* exposure) | *Lytechinus variegatus* (sperm) | no effects on mitochondrial membrane potential of sperm | [119] |
| Glyphosate | 10, 25, 50, 100, 150, 200 µg/L (*in vivo* exposure) | *Paracentrotus lividus* (larvae) | no abnormal development of larvae | [136] |
| **Corals** | | | | |
| Glyphosate + Increased temperature | 0.12, 1.2, 6, 12 mg/L (*in vivo* exposure) | *Acropora formosa* (adults) | significant effects of temperature*glyphosate interaction on coral pigmentation and chlorophyll *a* | [137] |
| **Polychaetes** | | | | |
| Roundup | from 0.065 to 65 mg/L (*in vivo* exposure) | *Laeonereis acuta* (adults) | LC50 determination<br>no significant change in oxygen consumption<br>significant decrease in AChE activity<br>significant decrease in ROS production after 24 h<br>significant decrease in antioxidant capacity against peroxyl radicals after 24 h<br>no significant alterations in antioxidant enzyme activities, except for SOD<br>significant alterations in lipid peroxidation | [138] |

Gametes and early life stages of echinoderms are widely considered as important targets to evaluate the effects of contaminants on the reproductive success of such invertebrates. In this regard, exposure of sperm from the sea urchin *Lytechinus variegatus* to different concentrations (0.25, 1, 4, and 16 mg/L) of Roundup Ready To-Use-Plus® did not cause significant alterations in mitochondrial membrane potential of sperm samples stained with MitoTracker. Sperm from only one animal treated with 16 mg/L showed a statistically significant variation in membrane integrity [119]. At concentrations ranging from 0 (control) to 200 µg/L, glyphosate did not affect significantly larval development of the sea urchin *Paracentrotus lividus* [136]. In addition, it was not possible to determine $EC_{50}$ (median

effective concentration) value, suggesting that glyphosate was not toxic to *P. lividus* larvae, at the concentrations tested at least [136].

In the ecotoxicological context, a growing concern is because the effects of contaminants can be exacerbated by variations in abiotic environmental factors, such as temperature. In this regard, Amid et al. [137] evaluated the combined effects of glyphosate and increased temperature to the tropical staghorn coral *Acropora formosa*. Branches of corals collected from both polluted and reference sites were exposed to different concentrations of glyphosate (0.12, 1.2, 6, and 12 mg/L) under two temperatures values, 28 °C ambient (ambient) and 31 °C (elevated temperature), and the effects on chlorophyll *a* content, zooxanthellae densities, and degree of bleaching were evaluated. Before exposure to increased temperature and glyphosate, corals from the reference site underwent bleaching, whereas those from the polluted site were more tolerant. Neither temperature nor glyphosate alone affected significantly coral pigmentation and chlorophyll *a*, whereas their interaction (temperature*glyphosate) had a significant effect on loss of color and chlorophyll *a*, mainly at the highest temperature and glyphosate levels. Results suggested that bleaching of corals can be promoted by different stressors occurring concomitantly.

Lastly, there is only one study in which a polychaete species was used to test glyphosate toxicity, to our knowledge at least. The species is the estuarine polychaeta *Laeonereis acuta* [138]. First, in that study the 96-h $LC_{50}$ value for Roundup was determined, it resulting to be 8.19 mg/L. Animals were then exposed for 24 h and 96 h to 3.25 mg/L (corresponding to the no observed effect concentration (NOEC)) and 5.35 mg/L ($LC_{10}$) and oxygen consumption of animals, as well as various biomarkers were measured in three body regions (anterior, middle and posterior) of polychaetes. Roundup did not cause significant alterations in oxygen consumption, whereas exposure induced a significant decrease in AChE activity in animals exposed to both Roundup concentrations. Significant reductions in reactive oxygen species (ROS) levels were recorded only in the posterior region of animals exposed for 24 h to Roundup concentrations, whereas a significant decrease in the antioxidant capacity against peroxyl radicals (ACAP) was observed in the three body regions of animals following exposure to the highest concentration of Roundup. No significant alterations in CAT, GST, and glutathione peroxidase (GPx) enzyme activities, along with reduced glutathione (GSH) levels, were recorded after exposure to Roundup. Conversely, superoxide dismutase (SOD) activity varied significantly depending on both animal regions and Roundup concentrations. Lastly, lipid peroxidation decreased significantly in all regions of animals exposed for 24 h to the highest concentration tested, whereas lipid damage generally increased in middle and posterior regions of polychaetes exposed for 96 h. Overall, results of that study demonstrated that Roundup can be seriously toxic to *L. acuta*, as alterations in ROS and ACAP levels, as well as in AChE activity demonstrated [138].

## 3. Conclusions and Perspectives

Although information concerning the levels of glyphosate in the marine environment is limited, the results of the studies that have been summarized in this review clearly indicate that this substance can cause undesirable effects on marine organisms, at different levels of biological organization. The growing interest in the potential risk posed by glyphosate to human health has also played an important role in the development of ecotoxicological studies aimed at understanding the negative effects of such substance to non-target organisms. Considering that the use of this herbicide is still permitted in many countries, further studies are necessary for a more in-depth assessment of the risk that glyphosate and its commercial formulations can pose to non-target marine organisms. In this context, it is important to highlight that data obtained in acute toxicity tests (few hours) indicate that glyphosate and its commercial formulations are generally lethal at high levels, that are not environmentally realistic. Conversely, information obtained following more prolonged exposure (several weeks) suggest that glyphosate can markedly affect biological responses of marine invertebrates. Consequently, efforts should be addressed at evaluating chronic or sub-chronic effects of such substances to other species of marine invertebrates.

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