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Commentary

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Contaminants of emerging concern and aquatic organisms: the need to consider hormetic responses in effect evaluations

Evgenios Agathokleous[1], Damià Barceló[2,3], Despo Fatta-Kassinos[4], Michael N. Moore[5,6,7], Edward J. Calabrese[8]

[1]School of Applied Meteorology, Nanjing University of Information Science and Technology (NUIST), Nanjing 210044, Jiangsu, China.
[2]Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Barcelona 08034, Spain.
[3]Catalan Institute for Water Research, ICRA-CERCA, Girona 17003, Spain.
[4]Department of Civil and Environmental Engineering and NIREAS-International Water Research Center, University of Cyprus, Nicosia 1678, Cyprus.
[5]European Centre for Environment & Human Health (ECEHH), University of Exeter Medical School, Knowledge Spa, Royal Cornwall Hospital, Truro 5356, UK.
[6]Plymouth Marine Laboratory, Plymouth, Devon 01404, UK.
[7]School of Biological & Marine Sciences, University of Plymouth, Plymouth 1752, UK.
[8]Department of Environmental Health Sciences, Morrill I, N344, University of Massachusetts, Amherst, MA 01003, USA.

Correspondence to: Prof. Evgenios Agathokleous, School of Applied Meteorology, Nanjing University of Information Science and Technology (NUIST), 219 Ningliu Rd., Nanjing 210044, Jiangsu, China. E-mail: evgenios@nuist.edu.cn

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Abstract

Contaminants of emerging concern are widespread in the world's waters, raising concerns regarding their effects on living organisms. To evaluate the effects of and predict risks associated with such chemicals, dose-response studies are needed, while the nature of the dose-response relationship is critical for the outcomes of such evaluations. Here, we summarize the literature reporting hormetic responses of aquatic organisms to contaminants of emerging concern. Hormesis is a biphasic dose response encompassing stimulatory responses to low doses and inhibitory responses to high doses. We demonstrate that it occurs widely in numerous aquatic organisms exposed to a wide array of contaminants, including nano/microplastics, suggesting potential effects at doses/concentrations that are considerably lower than the traditional toxicological threshold, which cannot be identified or predicted unless hormesis is considered in the study design. To tackle the effects and associated risks of nano/microplastics and other contaminants on aquatic organisms, hormesis should therefore be taken into account early in the design of studies as well as in relevant risk assessments.

Keywords: Dose-response relationship, ecological effects, ecotoxicological testing, environmental pollution, environmental management, hormesis, regulatory risk assessment, water contaminants

The liquid form of water not only is essential for human health[1] but is also fundamental for carbon-based life, i.e., the form of life on Earth that we currently know[2]. Even though two thirds of the surface area of Earth is covered with oceans, only 3% of the Earth's water is freshwater[3]. Furthermore, 99% of the liquid freshwater represents groundwater, and, thus, only 1% of the freshwater volume is accessible in lakes, reservoirs, and rivers[3]. Seventy percent of freshwater is used for agriculture in most regions of the world[4]. An about 60% increase of food production between the mid-2000s and 2050 will be needed; however, the world's water demand is predicted to increase by 55%[4]. Hence, preserving water quality is of paramount importance for the sustainability of the biosphere.

While numerous chemicals are essential in modern life, their extensive use leads to contamination of the water cycle, which is expected to increase with age, health, growth, and living standards of humans[5]. Numerous new chemicals are introduced yearly from industry and commerce as well as from consumption in the urban water cycle[4]. A study estimated that only 330 million people, at most, had access to safe water in China by 2010, and three quarter of the rural water sources were not considered safe by national standards[6]. There are numerous potential sources of water pollution, such as urban streets, suburban development, wastewater treatment plants, factories, croplands, animal feedlots, and rural homes, leading to extensive contamination of natural systems too[4]. Water quality and availability are expected to decrease due to climate change, indicating an increased need for reclaimed water from wastewater treatment for multiple purposes, yet effluent treatment is limited in its ability to remove contaminants of emerging concern[5]. Therefore, it is profoundly important to understand the effects of such contaminants on living organisms as well as their ecological risks. The assessment of the effects and risks associated with contaminants requires dose-response studies, the outcomes of which depend on the nature of the dose-response relationship. Hence, this commentary is aimed at summarizing hormetic responses of aquatic organisms to contaminants of emerging concern.

The literature on (eco)toxicological research has been largely framed within a linear-no-threshold (LNT) perspective throughout the 20th century, leading to the wide adoption of the LNT dose-response model by agencies in the United States as well as around the world[7-9]. According to the LNT model, chemical-induced damage increases proportionally to the dose (Figure 1A; hereafter. dose refers to concentration, exposure, or amount unless specified otherwise). LNT assumes that: (1) the damage is cumulative and irreversible; (2) there are no damage-repair mechanisms; (3) organisms lack any capacity to defend and protect themselves against environmental challenges; and (4) each single chemical molecule and the tiniest dose can produce damage up to some extent. The studies upon which the LNT is developed and widely adopted commonly include only few and typically very high doses that are usually environmentally irrelevant.

Along with LNT, the threshold model is one of the two most widely-used dose-response models in the scientific literature and broadly adopted in worldwide regulatory risk assessments for non-carcinogen chemicals of human health concern, and especially for ecological risk assessment[10-12]. The threshold model

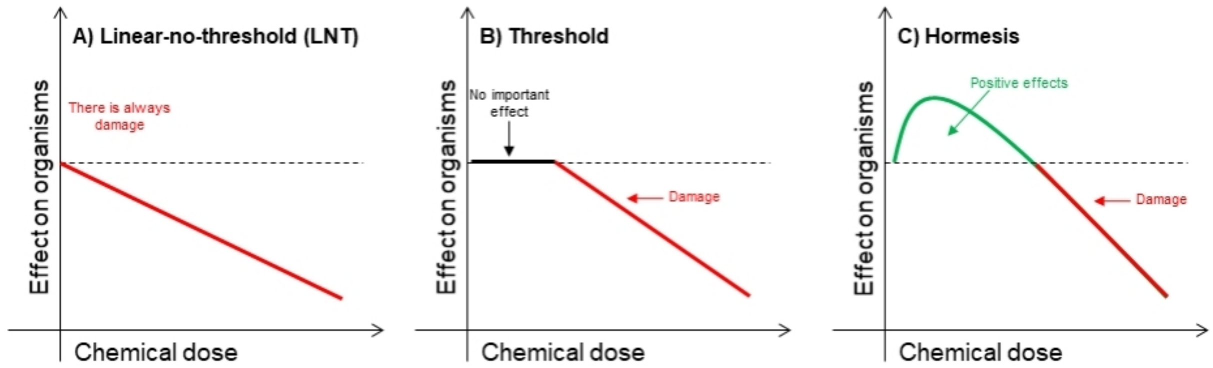

Figure 1. A hypothetical illustration of different dose responses: linear-no-threshold (A); threshold (B); and hormesis (C).

is characterized by an initially null response that extends up to a certain dose, which is considered as the toxicological threshold, and then followed by damage proportionally to dose similarly to the LNT model [Figure 1B]. Any potential effect of doses smaller than the toxicological threshold is attributed to random variance, due to, e.g., measurement, experimental, and/or environmental errors. Conversely to LNT, the threshold model refuses that each single molecule or the tiniest dose induces damage. It also denies the possibility that important responses of organisms can occur at doses smaller than the toxicological threshold.

In the recent years, however, hormesis has been brought into the mainstream of dose response science[13]. Hormesis is a dose response that is biphasic in nature, eventually having two distinct phases of responses/effects [Figure 1C]. The one phase represents responses that are commonly stimulatory and appears left to the no-observed-adverse-effect-level (NOAEL; toxicological threshold), whereas the other phase depicts negative effects (damage) and appears to the right of the NOAEL. Depending on the endpoint evaluated, it can be U- or J-shaped or their inverse[14]. Hormesis suggests that: (1) the damage is cumulative only after a threshold and is reversible; (2) there are damage-repair mechanisms; (3) organisms have the capacity to defend and protect themselves against environmental challenges; and (4) only doses larger than a threshold dose (NOAEL) can produce damage. It is therefore in striking contrast with the LNT model and to a lesser extent with the threshold model.

Hormesis was meant to climb the Hill at Golgotha from early in its inception as a scientific concept due to the - demonstrably incorrect - link with homeopathy, preventing its advancement throughout the 20th century[13]. Therefore, hormesis has scientifically matured mainly over the last two decades[13]. The current state of the art covers five major advancement areas: (1) documentation of its widespread occurrence and frequency in numerous organisms as a result of exposure to a plethora of chemical agents; (2) understanding of its generalized quantitative features (e.g., typically up to 60% stimulation) and their independency from the underlying mechanisms, stress-inducing agents, and organisms; (3) development of mathematical models for its statistical evaluation; (4) improvement of the understanding of the underlying mechanisms and potential associated ecological risks; and (5) recognition of its support by ecological and evolutionary theory[13-29].

Hormetic responses induced by aquatic contaminants/pollutants[1] in aquatic organisms have been documented since a long time ago. Pollutants are contaminants occurring at levels that can become hazardous to health and are commonly regulated. Readers may also refer to the Safe Drinking Water Act (https://www.epa.gov/ccl/definition-contaminant) for further information regarding the distinction between pollutants and contaminants. For instance, it was in 1981 that Laughlin *et al*.[30] reported in *Science* that low concentrations of petroleum hydrocarbons increased megalopal weight in zoeal mud crabs, effects that were opposite to the effects of high concentrations. There was however significant evidence for hormetic responses of aquatic organisms to pollutants earlier, such as the findings of Anthony Stebbing in a clonal hydroid exposed to metals[31,32]. Stebbing's work was seminal for the field, especially his 1982 review paper summarizing the hormetic responses of various aquatic and terrestrial organisms to different toxic agents and suggesting its generality[33]. It is thus clear that there was significant and broad evidence for this phenomenon since the 1980s. Some 30 years later, a review of the occurrence of hormesis in plants and algae in the literature, based on a database including nearly 700 dose-response curves and mathematical modeling, revealed the occurrence of hormesis in over 50% of the curves for the herbicides acifluorfen and terbuthylazine and in over 70% of the curves for glyphosate and metsulfuron-methyl[28].

Today, the evidence of hormesis induced by environmental pollutants and contaminants of emerging concern in aquatic organisms has considerably expanded. For example, a review of the literature concerning the effect of engineered nanomaterials on algae identified 46 hormetic-like dose responses[34]. Another detailed review of the published literature revealed an array of studies reporting hormetic responses of various terrestrial and aquatic creatures to micro- and nanoplastics[35], which are also supported by the findings of recent meta-analyses of the effects of microplastics on aquatic organisms, with significant hormetic responses to environmentally realistic concentrations, not only for genotoxicity but also for reproduction endpoints[36,37].

A further survey of the literature for the purpose of this study revealed ample evidence suggestive of hormesis for numerous aquatic organisms and various pollutants and emerging contaminants in dozens of publications, of which only a few selected examples are cited here (older examples can also be traced in the therein references). These examples include a plethora of species, such as of algae (microphytes)[38-49], aquatic flowering plants[50], organism-attached biofilms[51], crustaceans[52-57], cyanobacteria[49,58-61], fishes[62-69], macrophytes[70-75], marine polychaete[76], mollusks (e.g., clams and mussels)[77,78], periphyton[79], phytoplankton[80], sea anemones[81,82], and snails[83]. Responses suggestive of hormesis were found for molecular (molecules involved in oxidative stress), cellular (e.g., growth and density), and whole-organism (e.g., growth and body mass) endpoints, as well as endpoints suggesting potential sub-NOAEL effects on organismic interactions, such as via altered feeding activity[83]. Such responses were induced by chemicals such as antibiotics/antifungals[38,39,42,45,50,51,67,70,71,75], steroid hormones[41,83], and other human drugs[63,69], bisphenol A and its substitutes[66], chemical leached from disposed light sticks[55], electroplating process-emitted particulate matter[40], effluents from textile-dyeing wastewater treatment plants[46], fullerene crystals (nC_{60})[57], metals and ionic liquids[62,77,81,82], micro/nanoplastics and their leachates[43,44,47,53,60,64,80,84], engineered nanomaterials[58,59,61,65], pesticides[48,49,52,56,72-74,79,84], phthalic acid esters[78], and polybrominated diphenyl ethers[54]. These chemicals include many major contaminants of emerging concern and were applied either individually or jointly, indicating the potential occurrence of hormesis in aquatic organisms exposed to mixtures of such chemicals. Hormetic responses, however, are known to exhibit a significant temporal variation, including overcompensation stimulation, which suggests that a dose-time component needs to be further studied in relation to the response of aquatic organisms to contaminants of emerging concern in the future.

The literature survey and detailed review of the evaluated publications revealed that, in some key studies with findings of hormetic responses, hormesis was not acknowledged, while in other publications hormesis was claimed without sufficient evidence permitting such a conclusion. This suggests that, while hormesis acknowledgement is increased in the aquatic-related literature, there is still room for better understanding when it is present and when not. To tackle this issue and facilitate our understanding, it is suggested that researchers who are not immersed into the hormesis literature refer to some key review papers on the topic before drawing absolute conclusions on whether their results reflect hormesis.

In the light of the accumulated evidence for widespread occurrence of hormetic responses across aquatic organisms, hormetic response should be considered when evaluating the effects of micro/nanoplastics and other contaminants of emerging concern on aquatic organisms as well as in ecological risk assessments[85]. While the implications of hormetic responses to such contaminants for populations or communities of aquatic organisms are completely unknown, more information about the low-dose, sub-NOAEL effects is needed in order to feed future regulatory risk assessments and enhance the scientific understanding of the underlying mechanisms in aquatic organisms.

The herein analysis also indicates the need for improved research designs that will have the capacity to identify or predict hormetic responses, especially by including a higher number of doses in the sub-NOAEL zone. The maximum stimulation increases by 7% for each dose additionally included in the sub-NOAEL zone[86], indicating that the number of doses included in the sub-NOAEL zone affects the effect estimates. To minimize error from underestimation of sub-NOAEL effects, a minimum of six doses should be included in the sub-NOAEL zone[86]. Care should be exercised to include sub-NAOEL doses differing by orders of magnitude between them. Furthermore, sub-NOAEL responses are modest in amplitude, commonly not greater than 60% different from the control response[86], which makes their statistical detection difficult[19]. Hence, it is important that studies directed to study hormetic responses have robust experimental design with enhanced statistical power, indicating the need for a sufficient number of samples and/or experimental units. Finally, the temporal variation in the hormetic responses indicates the need for multiple assessments over time. The employment of a dependent-samples design with multiple levels could further improve the statistical power.

Although there is a perspective for developing chemicals exhibiting quick and complete degradation in the environment[5], a more rapid degradation in the environment might lead to higher exposures of living organisms due to spontaneously higher levels of bioavailable contaminants. Such spontaneous exposures, whether leading to positive or negative responses of individual organisms, could have unpredicted ecological implications. This exposition, by demonstrating the wide occurrence of responses to sub-NOAEL responses that are also more environmentally realistic, reinforces the need that agendas targeting chemical safety extend to natural ecosystems[87].

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Authors' contributions

Reviewed literature, drafted the manuscript, had a leading role, served as the hub of communication among the authors, and supervised the production of the manuscript: Agathokleous E

Reviewed the manuscript and contributed intellectual input: Barceló D, Fatta-Kassinos D, Moore MN, Calabrese EJ
Checked and approved the final version for publication: Agathokleous E, Barceló D, Fatta-Kassinos D, Moore MN, Calabrese EJ.

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