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Review

Environmental Aspect Concerning Phthalates Contamination: Analytical Approaches and Assessment of Biomonitoring in the Aquatic Environment

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Abstract: This review is a survey of recent progress in studies concerning the impact of phthalic acid esters in aquatic organisms. After introducing the classification, properties, sources, fate, and toxic effects related to phthalates, an overview of the techniques of extraction and analysis of these substances is provided. As a result, the general concepts of environmental bioindicators, biomonitoring systems, and other concepts related to phthalate contamination in the aquatic environment are presented. Recent bioaccumulation data of different phthalates are summarised in a table and organised according to the type of organism, tissue, and geographical area of sampling. Bioindicator organisms that are more representative of the different phthalates are highlighted and discussed as along with other variables that may be relevant in the assessment of the environmental pollution of these substances. The final part looks at the environmental perspectives and suggests new directions and research objectives to be achieved in the future.

Keywords: phthalic acid esters; endocrine active substances; extraction techniques; analytical method; biomonitoring; bioindicator

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1. Introduction

Phthalic acid esters (PAEs), commonly named phthalates, are a class of dialkyl or alkyl/aryl esters of phthalic acid (1,2-benzenedicarboxylic acid) structured in one benzene ring linked with two aliphatic ester groups, most commonly in the ortho configuration [1,2]. PAEs were used for the first time as additives in plastics in the 1920s and continue to be the largest plasticiser class in the 21st century [3]. Among all the possible sources of contamination, the impact of plastics in different environmental matrices has contributed to the widespread presence of phthalates. The release of chemicals associated with plastics into the marine environment is receiving increasing attention. Phthalates are biologically active compounds that dissolve in water to varying degrees depending on the physicochemical characteristics of the side chains, particularly octanol/water partitioning (K_{ow}). Organisms can absorb these substances by ingestion, inhalation, or contact [1].

In the organisms, PAEs are metabolised into toxic compounds that can impair vital functions. Di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP) are two of the most toxic and frequently used phthalates [4].

Animal experiments have shown that phthalates interfere with normal physiological processes mediated by hormones essential for reproduction, growth, and development (e.g., decreased testis weight, spermatogenesis impairment, and external genital malformations), leading to the so-called "phthalate syndrome" [5].

Based on the concentration, the nature of the compound, the physicochemical parameters of the environment, and the organism involved, exposure to PAEs leads to different effects and levels of chronic and acute toxicity [6].

Exposure to PAEs also adversely affects the behaviour and health of adults and their offspring [7,8] causing, among others, hepatotoxicity, oxidative stress, neurodevelopmental changes, genetic aberrations, and epigenetic reprogramming [7,9–12]. Depending on effects and exposure levels, phthalates can be considered risk factors for many multifactorial diseases (e.g., reproductive pathologies, developmental alterations and embryogenesis, including the hatching success of eggs, metabolic syndromes, and tumours) [7,13]. These effects are symptomatic of a hormone balance disorder; therefore, phthalates are endocrine active substances (EAS) that can interact or interfere with normal hormonal action, showing effects of different types and severity. For this reason, they can be called modulators, perturbators, disruptors, or endocrine destroyers.

In general, EAS can act in several ways: (i) mimic the action of the hormone naturally produced, inducing an excessive response or at the wrong times (agonistic effect); (ii) block the receptor, preventing the hormone from binding there so that it cannot act (antagonistic effect); (iii) alter the regulation of hormones, acting "upstream" on their production; (iv) alter the transport of hormones in the blood [14].

PAEs are substances of concern, as reiterated in the 2021 UN report on plastic pollution [15]; consequently, restrictive measures have been introduced, limiting their use.

The regulations on the restrictions on the use of phthalates are different between international legislations; moreover, they consider only phthalates with high rates of application, and thus, high risk of exposure, which are listed as toxic, for example, di-methyl phthalate (DMP), benzyl butylphthalate (BBzP), DEHP, DnBP, di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP), and di-n-octyl phthalate (DnOP). The restrictions mainly concern food contacts materials (FCM), cosmetics, toys, and childcare articles [2,16–18].

As published in the report of the European Chemical Agency (ECHA) (ANNEX XVII TO REACH—Conditions of restriction) from 7 July 2020, four phthalates (DEHP, BBzP, DnBP, and diisobutyl phthalate (DiBP)) cannot be placed on the market and cannot be used as individual substances or in combination in a concentration equal to or greater than 0.1% by weight of the plasticised material [18].

Considering the risks of exposure and the ubiquitous spread of these substances—that is likely to increase due to plastic pollution and is expected to double by 2030 [15]—monitoring activities should become routine and extensive to ensure the good health of affected ecosystems and affected organisms.

After introducing the main health risks and restrictions related to phthalates, the purpose of this review is to: (i) report the classification, properties, sources, and fate of PAEs; (ii) indicate the most common extraction and analysis methods in phthalate research; (iii) provide biomonitoring definitions and show which bioindicators revealed the higher concentration of each PAE that can be representative of different PAE contamination; and (iv) describe which variables may be relevant in the environmental assessment. Finally, a paragraph on the environmental perspectives suggests new research directions and objectives to be achieved in the future.

2. Physical, Chemical, and Environmental Properties of Phthalates

Phthalates are formed by a reaction of phthalic anhydride with various alcohols. The number of carbon atoms present will determine the length of the lateral chains R and R', and thus, the molecular weight of the phthalate is obtained [1]. PAEs differ chemically in the substitutions of the R1 and R2 side chains (which characterise their physicochemical properties) and are slightly volatile liquids, generally colourless, odourless, and oily liquids

at room temperature [6]. In addition, their solubility in fat (lipophilic property) increases with the lengthening of the side chains R and R′ (Figure 1).

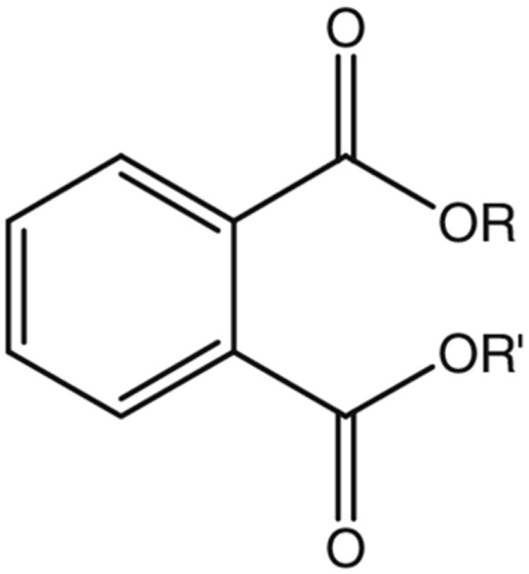

Figure 1. General chemical structure of phthalate esters.

Although there is no unique classification, it is generally possible to distinguish low molecular weight PAEs (LMW PAEs) with 3–6 carbon atoms in their side chain, and high molecular weight PAEs (HMW PAEs) with R and R′ from 7 to 13 carbons [1].

LMW PAEs include DMP, diethyl phthalate (DEP), DnBP, DiBP, and dimethylglycol phthalate (DMEP) and are typically used in PVC products, medical devices, personal care products, cosmetics, adhesives, paints, printing inks, pesticides, toys, enteric-coated tablets, food packaging or bag, etc. Most of the common phthalates are reported in Table 1.

PAEs with shorter alkyl chains, such as DMP and DEP, are widely used as solvents and fixatives, allowing fragrances, for example, to evaporate more slowly and to persist, thus extending product life [1,2,19]. HMW PAEs include BBzP, DEHP, DnOP, DiNP and DIDP, and dipropyl heptyl phthalate (DPHP), which are most commonly used as plasticisers to provide the plastic vinyl its flexibility [1].

PAEs have a relatively high boiling point and low melting point, which confers properties particularly suitable for use as plasticisers, heat transfer fluids, and carriers in the polymer industry [1]. Linear esters offer superior flexibility at low temperatures and have lower volatility than branched esters [1].

As a result of these characteristics, PAEs are widely used both as plasticisers and also as non-plasticising agents [20] in large quantities. In fact, some products may consist, by weight, of up to 40% of phthalates [21]. Despite their favourable physicochemical properties and their versatility of application in several fields that have provided numerous benefits to society, PAEs have instead demonstrated several adverse health effects of exposed organisms in all environments, especially in aquatic ones [22].

Since phthalates are not chemically bound to the polymers in which they are mixed, they can be released (for example, by contact, leaching, migration, or evaporation) in the environment, leading to exposure to the organisms present therein [22–24]. PAE residues have been detected in all environmental compartments. Extensive production, the storage of waste containing PAE in the environment, the inefficiency of traditional waste plants on the complete degradation, and the possible negative effects of PAEs on human health pose great global environmental and health risks for long durations [25].

Different reservoirs depend on different physicochemical properties, including water solubility (S_w), vapour pressure (V_p), Henry's constant (K_H), air/water partitioning, octanol/air partitioning (K_{oa}), octanol/water partitioning (K_{ow}), organic carbon partitioning (K_{oc}), and abiotic degradation/biodegradation processes [1].

In the aquatic environment, among all the possible sources of contamination, certainly, the impact of plastics (a major source of contamination) in different environmental matrices has contributed and is contributing to their ubiquitous diffusion (due to their ability to float and resist degradation). Phthalates, favoured by the size of micro- and nano-plastics, can easily pass from low trophic levels of the food chain such as plankton and fish and then up to top predators and humans [26].

In addition, it has been shown that microplastics [27] and therefore PAEs [9] can pass through the placenta, causing exposure of the foetus to these pollutants.

PAEs' presence has also been documented in regions far from the production areas due to the atmospheric and oceanic transport that contributes significantly to their spread [28]. This is particularly the case for short-chain phthalates, which are more susceptible to long-distance transport phenomena. As a result, they can also be found all over the world in regions where they have never been used or produced and it is very difficult to trace the source of origin [28]. To this, bioaccumulation and trophic transfer phenomena are added, further amplifying their diffusion.

The PAEs' fate and toxicity are correlated with the wide variety of environmental and biological transformations in different compartments, which depend on the structure and the physicochemical properties of the specific PAEs, the chemical nature of the investigated matrix, as well as different environmental conditions, including organic carbon content, pH, salinity, enzyme activities, etc. [29–31]. PAEs, similar to other persistent organic pollutants (POPs), are subject to biomagnification phenomena with potential negative impacts on the food chain, human health, and the environment [32].

Table 1. Most common phthalates with acronyms, molecular formulas, CAS, R1, and R2 chains and their log K_{ow}.

PAE Congeners	Acronym	Molecular Formula	CAS	R1	R2	Log K_{ow}
dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	131-11-3	CH_3	CH_3	1.60
diethyl phthalate	DEP	$C_{12}H_{14}O_4$	84-66-2	CH_2CH_3	CH_2CH_3	2.47
diisobutyl phthalate	DiBP	$C_{16}H_{22}O_4$	84-69-5	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	4.11
dibutyl phthalate	DnBP	$C_{16}H_{22}O_4$	84-74-2	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	4.50
dimethylglycol phthalate	DMEP	$C_{14}H_{18}O_6$	117-82-8	$CH_2CH_2OCH_3$	$CH_2CH_2OCH_3$	1.11 *
benzyl butyl phthalate	BBzP	$C_{19}H_{20}O_4$	85-68-7	$CH_2C_6H_5$	$CH_2C_6H_5$	4.73
dicyclohexyl phthalate	DCHP	$C_{20}H_{26}O_4$	84-61-7	$CH(CH_2)_5$	$CH(CH_2)_5$	5.6
di-n-pentyl phthalate	DnPP	$C_{18}H_{26}O_4$	131-18-0	$CH_2(CH_2)_3CH_3$	$CH_2(CH_2)_3CH_3$	5.62
bis (2-n-butoxyethyl) phthalate	DBEP	$C_{20}H_{30}O_6$	117-83-9	$CH_2CH_2O(CH_2)_3CH_3$	$CH_2CH_2O(CH_2)_3CH_3$	4.06 *
diphenyl phthalate	DPhP	$C_{24}H_{38}O_4$	84-62-8	C_6H_5	C_6H_5	n.a.
di(2-ethylhexyl) phthalate	DEHP	$C_{20}H_{14}O_4$	117-81-7	$CH(CH_2)_5(CH_3)_2$	$CH(CH_2)_5(CH_3)_2$	7.60
di-n-octyl phthalate	DnOP	$C_{24}H_{38}O_4$	117-84-0	$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	8.10
diisononyl phthalate	DiNP	$C_{26}H_{42}O_4$	28553-12-0	C_9H_{19}	C_9H_{19}	8.8
dinonyl phthalate	DnNP	$C_{26}H_{42}O_4$	84-76-4	C_9H_{19}	C_9H_{19}	9.52 *

Log K_{ow} values were obtained from PubChem [33]; when the calculated value was not present, the estimated value was added *; n.a.: not available.

The danger of phthalates derives from their ability to interact with cell membranes, which is justified by their affinity towards organic portions. This property can be represented by the partition coefficient octanol/water, log K_{ow}, i.e., the concentration ratio of a solute between octanol and water. K_{ow} provides an estimate of the hydrophobicity of a given molecule and can predict the tendency of the breakdown of a chemical in water, lipids, sediments, and soil organic matter.

3. Extraction and Analytical Methods

Over the past two decades, the growing interest of the scientific community and the need for improvements in the field of analytical detection led to the development of numerous extraction and analysis techniques to study an increasing number of phthalates [34] in different matrices.

For the correct assessment of phthalate concentrations in the different environmental matrices, the extraction and analysis methods should be sensitive and robust. Among the various difficulties encountered in their determination, an important aspect that deserves to be stressed are the problems of cross-contamination and the contamination of blank connected with the different processes of extraction and analysis [35].

The ubiquity of phthalates, especially of DEHP and DnBP, interferes with the determination of the phthalates to be studied, making some measures necessary to eliminate or minimise false positives [36].

Particular attention must be paid to all that are used and that can also come into contact with phthalates through vapours and particulates present in the working environment that can deposit or adhere to apparently uncontaminated objects [35].

In this context, all the tools used should be suitable for their use (for example, glass and ceramics) and should be properly cleaned with different rinsing cycles using solvents (for example, acetone and hexane) and kept dry at high temperatures [37,38]. At the same time, the most exposed analytical components, such as the injection needle, should be cleaned properly [35].

In order to ensure data quality, specific QA/QC protocols must be optimised. In this context, blank samples and blind samples should be used for each sampling campaign. Moreover, organic solvent used for extraction and calibration curves should be analysed to check possible cross-contaminations.

Finally, a quality control (QC) sample of secondary origin must be used to check both PAEs' degradation and contamination from the external.

3.1. Extraction Techniques

Different types of processes are used according to the type of matrix to be investigated in the sample pretreatment and phthalate extraction process. These extraction techniques can be resumed as solid–liquid extraction, solid-phase extraction, liquid–liquid extraction, and various others, as well as hybrid techniques [34,35,39].

Solid–liquid extraction (SLE) is a technique in which phthalates are extracted from the solid matrix through a solvent. This type of extraction can be performed by Soxhlet apparatus, ultrasonic bath, or mixing elution. Soxhlet is a technique that allows the extraction of analytes from solid materials used when the compound to be extracted has a limited solubility in the chosen solvent and the impurities are insoluble in it [35].

This extraction is not very common for the determination of phthalates and has the advantage that it does not need to be continuously monitored; in addition, it saves the solvent (e.g., cyclohexane, dichloromethane, or methanol) as the latter circulates continuously in the chamber to perform the extraction process [35]. However, the disadvantage lies in the long period of time required for the extraction process, which can be reduced with the temperature rise [35,40].

Unfortunately, in the case of complex organic matrices, the process may result in a high amount of analytical interference due to the matrix effect, and consequently the low recovery of analytes [41]. Moreover, on column, purification processes can be required.

Regarding liquid analyses, to date, solid-phase extraction (SPE) and liquid–liquid extraction (LLE) are used and further developed to promote extraction quality and cleanliness [35].

Solid-phase extraction (SPE) is the technique of extraction, purification, and concentration of analytes, best known and used for chemical analysis in different sectors (clinical, environmental, pharmaceutical, and food). The extraction process is based on the interaction of analytes to be extracted (affinity difference between analyte and interference),

present in a matrix/liquid phase with the solid phase called adsorbent (usually polymer matrix) present in the cartridge [42].

This technique saves time and solvents and can be prepared in semi- or full automation. Thanks to these advantages and practical operation, it is widely used, especially for water samples, in which the activated solid phase is used to extract PAEs from water samples and eluted with organic solvent [34,43].

In particular, the process can be divided into a preliminary phase of filtration of the water sample followed by four steps to be carried out in the cartridges: conditioning/equilibration, load (sample addition), washing, and elution [37]. The cartridges used for PAEs' extraction generally consist of short columns (made of polyethylene (PE) or polypropylene (PP)) containing sorbents (such as C18, octadecylsilane (ODS), HLB, etc.) that can have different sizes of porosity (usually 50–60 μm) [34].

Another type of SPE is the magnetic SPE, a low environmental impact technique with good sensitivity that uses a solution of a magnetic carbon nanotube based on dispersed iron. This technique can be automated in combination with gas chromatography, favouring low LOD (3.1–37 ng/L for 16 PAEs) [44]. Another similar technique called the dispersive graphene SPE (DSPE) uses graphene as a nanomaterial or a false adsorbent with a microsphere, printed with a magnetic molecule (MAG-MIM), in which organic desorption solvents are usually acetonitrile, acetone, ethyl acetate, and n-hexane [45].

Solid-phase microextraction (SPME) is also considered a green technique as it is solvent-free and includes the absorption of analytes on a microfiber coated with a hydrophilic polymer, such as polydimethylsiloxane/divinylbenzene (PDMS/DVB), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and polyacrylate (PA) fibres [46]. However, a few studies in the literature use this technique (also online, with mass analysers), so it is still under development and has been shown to be effective only for some phthalates [34].

Stir bar sorptive extraction (SBSE) is another green method based on the extraction of organic compounds such as phthalates from aqueous samples without the use of solvents or concentration phases. The method consists of the extraction of solutes by adsorption in a polymer made up of PDMS that covers a stir bar [34,47].

Solid-phase extraction, liquid–liquid extraction (LLE), and LLE-similar are the most widely used methods for the determination of phthalates [35].

Liquid–liquid extraction is a method used for the extraction of analytes in aqueous samples by organic solvent, for example, hexane and dichloromethane (DCM) [34,48]. Shaking the mixture vigorously promotes the extraction of analytes. After decantation, a separation will be obtained between the aqueous phase and the organic phase (not miscible), containing phthalates that will then be collected and analysed while the aqueous solution can be subjected again to the process to improve the recovery of the analytes [49]. In addition, it is possible to add organic modifiers such as methanol to have a better extraction efficiency of most non-polar PAEs such as DEHP and DnOP [50], and the separation process can be improved by coupling sonication, centrifugation, and freezing [34]. Despite the ease of application, the LLE procedure requires a large amount of organic solvents with environmental and economic consequences [51].

Solid-supported LLE (SLE), considered a hybrid method between SPE and LLE, is a technique in which an aqueous sample initially interacts with a cartridge divided with a sorbent in diatomaceous earth that retains both analytes and matrix components, subsequently, the PAEs are then selectively eluted through into an immiscible organic solvent [52].

A similar method using very low volumes (a few microlitres) of immiscible substances is called liquid-phase microextraction (LPME), which can be combined with different extraction processes that maximise its effectiveness [34,52]. These microextraction techniques include single-drop microextraction (SDME), hollow-fibre LPME (HF-LPME), dispersive liquid–liquid microextraction (DLLME) and its different forms (ultrasound-assisted dispersive liquid–liquid extraction (UA-DLLME), ultrasound-vortex-assisted dispersive liquid–liquid microextraction (USVA-DLLME), and magnetic stirring-assisted dispersive

liquid–liquid microextraction (MSA-DLLME)), and cold-induced aggregation microextraction (CIA-ME) [34].

Another method that takes advantage of the distribution balance of the two phases is the microporous membrane LLE (MMLLE). This method allows the automation of the micro-LLE process on a PP membrane that permits the entry of the aqueous phase together with the organic solvent [50]. Following the same chemical principle in the homogeneous liquid–liquid extraction (HLLE), the area of contact between two phases (water and organic solvent) is extremely large, consenting a rapid attainment of repartition equilibrium [34].

Among the increasingly used methods for the determination of pollutants, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) technique has taken significant importance in recent years [53].

The QuEChERS procedure involves a series of methods aimed at solving all problems due to the heterogeneous nature of the sample (for example, long extraction times due to several passages, large quantities of solvents to be used, etc.) [53–55] and can be used to extract compounds from a solid and liquid matrix. The process consists of the transfer of analytes from solid or liquid samples to the extraction phase. After sample homogenisation, an initial extraction with acetonitrile is carried out, followed by a phase of buffer salts' addition and centrifugation [54]. Then, the supernatant will be purified (removing the interferents) through the dispersive solid-phase microextraction (d-SPME) [53,54]. This method can be optimised by modifying some of the parameters that affect extraction efficiency, such as extraction solvent type and volume, sample quantity, pH, salt selection, etc. [54]. Another simple and fast technique of PAEs' extraction is accelerated solvent extraction (ASE); this method is based on the use of high temperatures (above the solvents' boiling points) and pressures and allows a greater efficiency of the extraction of analytes from the matrix [52]. An alternative extraction technique for solid matrices is thermodesorption (TD); this green technique does not require sample preparation treatments and allows a direct analysis of the sample that is heated vaporised and analysed with high reproducibility and quality of results [52].

A summary of the most important techniques used for PAEs from environmental matrices is reported in Table 2.

Table 2. Summary of the most used extraction method for PAEs from environmental matrix.

Phthalates Extraction in Different Environmental Matrices	Extraction Methods	Type of Extraction Procedures
Water	Liquid–liquid extraction (LLE)	Separation funnel Ultrasound/vortex to assist LLE
	Liquid–solid extraction (LSE)	Solid-phase extraction (SPE) Dispersive solid-phase extraction (d-SPE) Solid-phase microextraction (SPME) Stir bar sorptive extraction (SBSE)
	Hybrid method	Solid-supported LLE (SLE) Liquid-phase microextraction (LPME) Single-drop microextraction (SDME), Hollow-fibre LPME (HF-LPME) Dispersive liquid–liquid microextraction (DLLME) Microporous membrane LLE (MMLLE)
Sediments and Biota	Solid–liquid extraction (SLE)	Ultrasound/vortex to assist SLE Soxhlet extraction Accelerated solvent extraction (ASE)
	Solid extraction (SE)	Thermodesorption (TD)
Water, Sediments and Biota	Quick, easy, cheap, effective, rugged, and safe (QuECHERS)	Combination of LLE and d-SPE

3.2. Analytical Method

After the sample pretreatment and the phthalates extraction, they are determined by quali/quantitative analysis using instrumental techniques which, together with the extraction techniques, determine the quality of the detection. The variables that regulate the choice of the appropriate analytical technique depend on the type of analyte and the instrumental sensitivity.

The most commonly used methods are gas chromatography (GC) and liquid chromatography (LC) coupled to detectors such as mass spectrometer (MS), which allows measuring the mass/charge ratio (m/z) of analytes [34]. Generally, MS detector is selected as the best detector system for these types of analyses; moreover, in the case of water samples, analyses by LC instrument can be performed without extraction or purification systems.

Gas chromatographic analysis is the most widely used separation technique for PAEs analysis, as phthalates have volatile and thermostable characteristics [52]. Generally, non-polar capillary chromatographic columns (in a thermostatic oven) and helium gas are used.

Gas chromatography coupled with MS has many advantages, such as a short analysis time, high resolution, and sensitivity [34]. With the MS detector, each PAE can be ionised by electronic impact (EI) and detected by full scan, single ion monitoring (SIM), selected ion storage (SIS), MS tandem (MS/MS), or multiple reaction monitoring (MRM), improving its sensitivity [34,52].

High-pressure liquid chromatography is a technique that allows the separation of two or more compounds present in a solvent, exploiting the affinity equilibrium between a mobile phase, in which phthalates are dissolved (mixture of liquid-pressurised solvents usually consisting of acetonitrile and water) and a stationary phase (absorbent material, typically granular silica or polymer) is placed inside the chromatographic column [53,56].

Phthalates can be analysed using liquid chromatography coupled to mass spectrometry (LC-MS) with electrospray (ESI), or atmospheric pressure chemical (APCI), and ionisation in positive mode [34,52].

Detectors such as diode array detector (DAD) are also used in the literature or UV coupled with LC, which have the advantage of being economic techniques with good performance where dissolved analytes can be recovered; however, these have slightly lower sensitivity than GC methods [57].

Notably, when comparing GC and LC results in PAEs analyses, the latter showed a lower sensitivity for major phthalates [34,52], while HPLC, and more recently, ultra HPLC (UHPLC) were more adequate for the analyses of PAEs' degradation products and for PAEs' isomeric mixtures (e.g., DiNP, DiDP) [52].

In fact, low detection limits in water samples for the following analytical techniques have been reported: LC-MS analysis of ten mono-alkyl phthalate esters (MPEs) of 0.19–3.9 ng/L; GC-MS analysis of five PAEs: 1.62–16.3 ng/L; and LC-UV analysis of three PAEs: 10–20 ng/L [52].

Among the mass spectrometry instrumentation combined with either the LC or GC system (quadrupole, triple quadrupole, ion trap, and magnetic sector), the triple quadrupole is the most frequently used for strength, sensitivity, and stability [52]. In addition, more recently quadruple hybrid systems associated with TOF and Orbitrap, and the latter in particular has excellent resolution, mass accuracy, sensitivity, and selectivity for phthalates [58,59].

Although less used, other chromatographic techniques replacing LC or GC for PAEs analysis are micellar electrokinetic capillary chromatography (MEKC), Fourier transform infrared spectroscopy (FTIR) and colourimetric analysis. Similarly, non-chromatographic methods are also less used than GC and LC and rely on recent molecular imprinting technologies and immunoassay-based techniques. The advantage of these techniques is that they have a lower maintenance cost and fewer blank contamination problems than LC and GC. However, these techniques are still in development [34,52,57].

4. Bioindicators and Levels of PAEs in the Aquatic Environment

The assessment of the pollution impact on the aquatic environment is of fundamental importance for the life of ecosystems and the connections between the biological systems involved.

"Biomonitoring" is a scientific technique for environmental status assessment that measures the health of an ecosystem using biological indicators (bioindicators) [60]. In general, the term biological indicator defines all sources of biotic and abiotic reactions related to changes in a given ecosystem in which taxa are used to identify the effects of changes in the environment [61]. Some organisms are used as an indicator to monitor contaminant uptake, bioavailability, excretion, and determination of toxic effects [60]. For the latter, at the experimental level, through model bioindicator organisms, it is possible to monitor the effects of substances to evaluate their health effects [32,60].

In the field of environmental chemical science, natural bioindicators or biomarkers are an important tool to detect environmental changes by evaluating the state of contamination based on their presence or absence, or quantitatively through their analysis. In this context, it is possible to distinguish pollution bioindicator (for detection of pollutants), ecological bioindicator (for evaluation of change of natural surroundings), environmental bioindicator (for assessing environmental changes), and biodiversity bioindicator (for monitoring the presence/absence of species present in it) [62].

For qualitative/quantitative analyses of pollutants, bioaccumulator/bioconcentrator organisms are a powerful and sensitive instrument also adopted for monitoring and environmental quality surveys [32,60]. These organisms are pollutant bioindicators that accumulate in their tissues at a level that exceeds that of the contaminated medium (e.g., water) and is the result of chemical absorption through all routes of exposure [32].

They allow the assessment of the bioaccumulation trend of chemical contaminants through the chemical analysis of their tissues, allowing for an indirect evaluation of the evolution of the contamination in the ecosystem [60]. They are also an economically viable alternative to other specialised measuring systems.

In general, an ideal organism for toxicological, biomonitoring, biodistribution, and bioaccumulation studies should meet certain characteristics, such as easy sampling, year-round availability, wide distribution, sensitivity or tolerance to pollutants, resistance to environmental variability, reduced mobility (e.g., sessile species for limited spatial contamination assessment), easy recognition (for example, species or sex), long life cycle for biomonitoring and bioaccumulation studies, or short life cycle for some experimental studies [32,61,63]. For the latter, it is important that the model organism has a rapid development, is easy to manage under controlled conditions, and that there is adequate knowledge of its physiological, genetic, and biomolecular mechanisms [62,64].

In detail, sentinel organisms are ideal bioindicators that provide prompt and early important information on the ecosystem health assessment and therefore on the presence of potential negative impacts. For example, marine mammals are defined as first sentinels because many of them are long-lived, are found at high trophic levels, have ecological habits that lead them to linger for a long time in the coastal areas subjected to a greater anthropic impact, and have large fat deposits that accumulate lipophilic chemicals such as POPs [65].

Understanding bioaccumulation processes is of considerable importance for several reasons: (i) bioaccumulation in the tissues of organisms may increase the persistence of chemicals in the environment; (ii) stored chemicals are not exposed to physical, chemical, or direct biochemical degradation; (iii) accumulated biologically active substances can directly affect the health of an individual; and (iv) predators of those contaminated organisms may be threatened by the effects of the toxic substances as they can, at their turn, accumulate such substances, even at higher concentrations (biomagnification) [66].

An ideal bioaccumulator is a bioindicator that, due to its characteristics, accumulates more pollutants than other species in the same environment. In this way, this sentinel organism will better reflect contamination levels even in areas where there is apparently no

human impact. As reported in the literature [67], contamination levels of water posed varied ecological risks to organisms and environment composition. Generally, the concentration levels found in water are three orders of magnitude lower than that found in sediment and biota; however, the predominant PAEs' composition congeners are similar, for example, the greatest contribution of total phthalates is usually provided by DnBP and DEHP [68,69] (see Table 3). Moreover, significant correlations were found between biota contamination levels and aqueous environment of biota origin [68].

In this context, some species bioaccumulate more than others, for example, due to their ecological behaviour (foraging/respiratory), which facilitates the assimilation of pollutants. Among these species, filter-feeding organisms are excellent models of biomonitoring (e.g., bivalves). Recently, for example, Mediterranean mussels (*Mytilus galloprovincialis*), organisms known to be bioindicators of environmental contamination, have been used as the sentinel species of pollution by plasticisers, including phthalates with excellent detection results [69].

Other eating habits, such as whales' 'bubble net feeding', lead to the accidental and massive ingestion of PAEs contained in plastic fragments and are one of the reasons why some organisms are more exposed to such dangerous substances [70]. For example, it has recently been observed that, among the blubber lipophilic constituents of the blue whale (*Balaenoptera musculus*), four phthalates were significantly represented [71].

Phthalates can also highly contaminate organisms because they are contained in the plastic material exchanged for prey (e.g., plastic bags mistaken for jellyfish by sea turtles [72]). Emblematic cases of this frequent ingestion is associated with sea turtles, considered excellent descriptors of marine pollution [73–76] and defined bioindicators for marine litter in the European Union Marine Strategy Framework Directive (MSFD) [77]. Recently, due to the high incidence of the discovery of plastic debris in the gastrointestinal tract of sea turtles, new evidences of major levels of phthalates in their tissues [78] and eggs [79].

Another frequent contamination process is related to the ingestion of prey that in turn had ingested plastic. This is now known for organisms at the top of the food chain (such as sharks) that therefore tend to accumulate pollutants due to biomagnification phenomena. In a case study in the Mediterranean Sea, the basking shark (*Cetorhinus maximus*) showed concentrations of mono-(2-ethylhexyl) phthalate (MEHP) (primary metabolite and DEHP exposure marker) in the muscles above the high values recorded in whale blubber (*Balaenoptera physalus*) [70].

These organisms deserve particular interest and concern because they are often included in the lists of threatened species. In this context, the direct effects of exposure to PAEs could have negative effects on their health (including reproductive success) affecting their conservation status. Moreover, these species, which best represent the environmental contamination of phthalates, allow us to deduce the incidence of these ubiquitous substances along the food chain, with consequent risks associated with the health of ecosystems and humans.

In aquatic environments, the concentration levels of PAEs recorded are variable, as the pollution associated with different sampling areas. In this context, within the same sampling site, a further variability is given by the type of bioindicator analysed based on physiological characteristics, trophic level, feeding behaviour, etc. Table 3 shows data on the concentration of the main phthalates industrially used and analysed for environmental biomonitoring in different classes of organisms.

In this table, the different concentration values of 16 phthalates appear to be related to the type of organism, the geographical area, and the type of tissue analysed.

Table 3. Concentration values of individual biomonitored phthalates and their total concentration in different aquatic organisms. The table is based on the type of organism, the species, the geographical area of sampling, and the type of sample analysed. For more details including acronyms and molecular formula, see Table 1.

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Actinopterygii	*Mullus barbatus*	Tyrrhenian Sea	gills	649.0	245.0		305.0							284.0	1061.0	1491.0		2544.0 (ng/g d.w.)	[80]
Actinopterygii	*Mullus barbatus*	Tyrrhenian Sea	muscles	191.0	97.0		101.0							776.0	103.0	144.0		1268.0 (ng/g d.w.)	[80]
Actinopterygii	*Acanthopagrus Schlegelii*	Xiangshan Bay (East China Sea)	muscles	61.1	9.5	397.8	364.3	11.9	35.3			16.3	6.6	253.0			4.4	1160.0 (ng/g d.w.)	[81]
Actinopterygii	*Arius maculatus*	Yangtze River Delta area (East China Sea)	muscles	0.9	4.5		n.d.							643.0	n.d.			648.4 (ng/g w.w.)	[82]
Actinopterygii	*Boleophthalmus pectinirostris*	Yangtze River Delta area (East China Sea)	muscles	1.4	0.1		21.8							133.0	n.d.			156.4 (ng/g w.w.)	[82]
Actinopterygii	*Centropristis striata*	Xiangshan Bay (East China Sea)	muscles	11.3	7.2	1938.0	659.0	8.6	30.5	4.2		94.9	1.8	2168.0	3.2			4926.7 (ng/g d.w.)	[81]
Actinopterygii	*Chelidonichthys spinosus*	Yangtze River Delta area (East China Sea)	muscles	0.8	3.7		n.d.							46.1	n.d.			50.7 (ng/g w.w.)	[82]
Actinopterygii	*Clupea pallasii*	Yangtze River Delta area (East China Sea)	muscles	n.d.	1.8		2.4							119.0	n.d.			123.2 (ng/g w.w.)	[82]
Actinopterygii	*Diplodus annularis*	Tyrrhenian Sea	gills	441.0	144.0		267.0							666.0	175.0	229.0		1693.0 (ng/g d.w.)	[80]
Actinopterygii	*Diplodus annularis*	Tyrrhenian Sea	muscles	231.0	96.0		108.0							187.0	186.0	200.0		808.0 (ng/g d.w.)	[80]
Actinopterygii	*Diplodus vulgaris Oblada melanura Serranus cabrilla Serranus scriba*	Cabrera MPA (Balearic Sea)	muscles		170.0		720.0							880.0				1770.0 (ng/g w.w.)	[69]
Actinopterygii	*Ditrema temmincki Bleeker*	Xiangshan Bay (East China Sea)	muscles	12.3	4.4	530.0	199.0			45.8				732.0				1523.5 (ng/g d.w.)	[81]
Actinopterygii	*Epinephelus akaara*	Xiangshan Bay (East China Sea)	muscles	7.3	7.3	854.5	351.0	9.1	11.3	42.2			1.3	337.0				1620.9 (ng/g d.w.)	[81]
Actinopterygii	*Epinephelus goreensis*	Xiangshan Bay (East China Sea)	muscles	18.8	12.9	541.0	369.5					7.6		246.0			1.4	1197.2 (ng/g d.w.)	[81]
Actinopterygii	*Eucyclogobiusnewberryi*	Yangtze River Delta area (East China Sea)	muscles	1.1	n.d.		n.d.							3.9	n.d.			5.0 (ng/g w.w.)	[82]

Table 3. *Cont.*

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Actinopterygii	*Gadus morhua*	local fishmonger (Tarragona, Spain)	muscles		11.4	n.d.								n.d.				12.7 (ng/g w.w.)	[83]
Actinopterygii	*Jordanella floridae*	Xiangshan Bay (East China Sea)	muscles	19.6	9.9	1661.0	323.0		74.2					347.0	6.3			2440.9 (ng/g d.w.)	[81]
Actinopterygii	*Konosirus Punctatus*	Xiangshan Bay (East China Sea)	muscles	145.0	13.0	35.7	672.0			9.6	53.0			201.0	51.8			1181.1 (ng/g d.w.)	[81]
Actinopterygii	*Larimichthys crocea*	Xiangshan Bay (East China Sea)	muscles	159.3	10.4	665.6	1025.5	9.2				43.1	2.4	540.6			2.2	2458.2 (ng/g d.w.)	[81]
Actinopterygii	*Larimichthys polyactis*	Yangtze River Delta area (East China Sea)	muscles	0.5	0.4		n.d.							68.8	n.d.			69.7 (ng/g w.w.)	[82]
Actinopterygii	*Lateolabrax Japonicus*	Xiangshan Bay (East China Sea)	muscles	25.0	17.4	493.7	262.7	15.0	17.2		2.7			685.7	2.3		8.2	1529.7 (ng/g d.w.)	[81]
Actinopterygii	*Lophius litulon*	Yangtze River Delta area (East China Sea)	muscles	0.6	1.3		4.9							161.2	n.d.			168.1 (ng/g w.w.)	[82]
Actinopterygii	*Merluccius merluccius*	local fishmonger (Tarragona, Spain)	muscles		9.0	1.3								n.d.				10.3 (ng/g w.w.)	[83]
Actinopterygii	*Mugil cephalus*	Xiangshan Bay (East China Sea)	muscles	119.4	10.0	698.5	858.5	13.6		16.4	27.8			276.0			3.1	2023.4 (ng/g d.w.)	[81]
Actinopterygii	*Mugil cephalus*	Yangtze River Delta area (East China Sea)	muscles	0.8	3.6		3.2							587.6	n.d.			595.2 (ng/g w.w.)	[82]
Actinopterygii	*Mugil cephalus*	Tyrrhenian Sea	gills	298.0	212.0		407.0							647.0	157.0	134.0		1721.0 (ng/g d.w.)	[80]
Actinopterygii	*Mugil cephalus*	Tyrrhenian Sea	muscles	182.0	86.0		132.0							316.0	59.0	116.0		775.0 (ng/g d.w.)	[80]
Actinopterygii	*Muraenesox cinereus*	Yangtze River Delta area (East China Sea)	muscles	0.1	0.3		n.d.							n.d.	n.d.			0.4 (ng/g w.w.)	[82]
Actinopterygii	*Nibea Albiflora*	Xiangshan Bay (East China Sea)	muscles	79.6	23.5	556.1	643.4	15.2	26.1			34.1	1.1	219.3			3.3	1601.6 (ng/g d.w.)	[81]
Actinopterygii	*Pagrus Major*	Xiangshan Bay (East China Sea)	muscles	74.0	11.4	288.5	655.5		23.1			13.9		1270.5				2336.8 (ng/g d.w.)	[81]
Actinopterygii	*Pampusargenteus*	Yangtze River Delta area (East China Sea)	muscles	0.9	3.0		n.d.							1941.0	1.5			1946.4 (ng/g w.w.)	[82]

Table 3. *Cont.*

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Actinopterygii	*Platycephalus indicus*	Yangtze River Delta area (East China Sea)	muscles	0.3	5.9		10.5							250.0	n.d.			266.7 (ng/g w.w.)	[82]
Actinopterygii	*Salmo salar*	local fishmonger (Tarragona, Spain)	muscles		61.0	n.d.								n.d.				61.0 (ng/g w.w.)	[83]
Actinopterygii	*Sarda orientalis*	Yangtze River Delta area (East China Sea)	muscles	0.9	3.3		43.6							281.1	n.d.			328.8 (ng/g w.w.)	[82]
Actinopterygii	*Sardina pilchardus*	local fishmonger (Tarragona, Spain)	muscles		102.1	n.d.								n.d.				323.8 (ng/g w.w.)	[83]
Actinopterygii	*Sciaemops ocellatus*	Xiangshan Bay (East China Sea)	muscles	32.5	3.8	1822.0	131.0	11.0		9.1	23.8		52.6	2069.0				4154.7 (ng/g d.w.)	[81]
Actinopterygii	*Scomber japonicus*	Yangtze River Delta area (East China Sea)	muscles	1.0	13.6		2.5							108.5	n.d.			125.6 (ng/g w.w.)	[82]
Actinopterygii	*Scomber vincialis*	local fishmonger (Tarragona. Spain)	muscles		2.9	n.d.								n.d.				2.9 (ng/g w.w.)	[83]
Actinopterygii	*Scomberomorus niphonius*	Yangtze River Delta area (East China Sea)	muscles	1.3	0.1		n.d.							51.6	n.d.			53.0 (ng/g w.w.)	[82]
Actinopterygii	*Solea solea*	local fishmonger (Tarragona, Spain)	muscles		15.0	1.0								8.3				27.5 (ng/g w.w.)	[83]
Actinopterygii	*Sphyraenus*	Xiangshan Bay (East China Sea)	muscles	10.9	36.9	1076.0	128.0					60.3	3.9	392.0	26.5			1734.5 (ng/g d.w.)	[81]
Actinopterygii	*Thunnus thynnus*	Yangtze River Delta area (East China Sea)	muscles	0.1	2.5		2.6							7.4	n.d.			12.6 (ng/g w.w.)	[82]
Actinopterygii	*Thunnus thynnus*	local fishmonger (Tarragona, Spain)	muscles		19.4	n.d.								n.d.				19.4 (ng/g w.w.)	[83]
Actinopterygii	*Trachinotus ovatus*	Xiangshan Bay (East China Sea)	muscles	13.9	7.4	1791.0	102.0	28.6					1.2	1148.0	10.0			3102.1 (ng/g d.w.)	[81]
Actinopterygii	*Trachurus japonicus*	Yangtze River Delta area (East China Sea)	muscles	0.7	2.5		n.d.							137.7	n.d.			140.8 (ng/g w.w.)	[82]
Actinopterygii	*Trichiurus lepturus*	Yangtze River Delta area (East China Sea)	muscles	0.01	1.3		n.d.							126.0	n.d.			127.3 (ng/g w.w.)	[82]
Actinopterygii	*Zeus faber*	Yangtze River Delta area (East China Sea)	muscles	0.5	4.2		n.d.							n.d.	28.1			32.9 (ng/g w.w.)	[82]
Ascidiacea	*Herdmania momus*	Mikhmoret beach (Mediterranean Sea)	whole body				5064.0							9095.0				14,159.0 (ng/g d.w.)	[84]

Table 3. *Cont.*

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Ascidiacea	*Herdmania momus*	Eilat marina (Red Sea)	whole body				3757.0							5556.0				9313.0 (ng/g d.w.)	[84]
Ascidiacea	*Microcosmus exasperatus*	Palmahim national park (Mediterranean Sea)	whole body				1643.0							4988.0				6631.0 (ng/g d.w.)	[84]
Ascidiacea	*Microcosmus exasperatus*	Bat-Yam beach (Mediterranean Sea)	whole body				2224.0							4851.0				7075.0 (ng/g d.w.)	[84]
Bivalvia	*Crassostrea virginica*	Florida coast (United States)	soft tissues	1.3	1.9		3.0			3.2				70.4	0.2			79.8 (ng/g w.w.)	[85]
Bivalvia	*Mussels*	Estuary of Bilbao (Spain)	soft tissues	132.1	391.1		1673.8			592.2				8355.6	37.3			11,182.1 (ng/g d.w.)	[86]
Bivalvia	*Mytilus galloprovincialis*	local fishmonger (Tarragona, Spain)	soft tissues		67.3	6.6								n.d.				73.9 (ng/g w.w.)	[83]
Bivalvia	*Ruditapes philippinarum*	Yangtze River Delta area (East China Sea)	soft tissues	0.7	1.0		3.8							270.5	0.8			276.8 (ng/g w.w.)	[82]
Bivalvia	*Sinonovacula constrzcta*	Yangtze River Delta area (East China Sea)	soft tissues	0.9	0.02		1.5							99.2	n.d.			101.7 (ng/g w.w.)	[82]
Bivalvia	*Arca noae*	Cabrera MPA (Balearic Sea)	soft tissues		540.0		780.0							2580.0				3900.0 (ng/g w.w.)	[69]
Cephalopoda	*Loligo vulgaris*	local fishmonger (Tarragona, Spain)	soft tissues		n.d.	n.d.								13.8				14.8 (ng/g w.w.)	[83]
Crustacea	*Aristeus antennatus*	local fishmonger (Tarragona, Spain)	soft tissues		36.9	n.d.								10.9				49.4 (ng/g w.w.)	[83]
Crustacea	*Penaeus chinensis*	Yangtze River Delta area (East China Sea)	soft tissues	0.4	1.7		11.2							93.0	n.d.			106.2 (ng/g w.w.)	[82]
Crustacea	*Solenocera crassicornis*	Yangtze River Delta area (East China Sea)	soft tissues	0.8	0.8		5.3							82.8	0.1			89.8 (ng/g w.w.)	[82]
Crustacea	*Talitrus saltator Parhyale plumicornis Parhyale aquilina, Speziorchestia stephenseni, Orchestia montagui*	Stagnone di Marsala—Sicily (Mediterranean Sea)	whole body		108.0	97.0	23.0							46.0				292.0 (ng/g w.w.)	[87]

Table 3. *Cont.*

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Gastropoda	*Bullacta exarata*	Yangtze River Delta area (East China Sea)	soft tissues	0.7	n.d.		9.6							179.0	n.d.			189.2 (ng/g w.w.)	[82]
Holothuroidea	*Holothuria forskali, Holothuria poli, Holothuria tubulosa*	Cabrera MPA (Balearic Sea)	muscles		490.0		1240.0							1480.0				3210.0 (ng/g w.w.)	[69]
Mammalia	*Balaenoptera physalus*	Iceland (North Atlantic Ocean)	muscles	8.0	303.0		303.0							10.0				624.0 (ng/g d.w.) *	[88]
Mammalia	*Globicephala macrorhynchus*	Macaronesian Region (Eastern North Atlantic)	muscles				969.0							335.1				1304.1 (ng/g w.w.)	[89]
Mammalia	*Grampus griseus*	Macaronesian Region (Eastern North Atlantic)	muscles		84.7		557.8							380.8				1023.3 (ng/g w.w.)	[89]
Mammalia	*Kogia breviceps*	Macaronesian Region (Eastern North Atlantic)	muscles				664.0							102.0				766.0 (ng/g w.w.)	[89]
Mammalia	*Kogia* spp.	Atlantic coast of North Carolina and Florida	blubber		200.0													200.0 (ng/g d.w.) *	[90]
Mammalia	*Lagenodelphis hosei*	Macaronesian Region (Eastern North Atlantic)	muscles		97.7		552.0							329.7				979.3 (ng/g w.w.)	[89]
Mammalia	*Lagenorhynchus albirostris*	Atlantic coast of North Carolina and Florida	blubber		13,800.0													13,800.0 (ng/g d.w.) *	[90]
Mammalia	*Peponocephala electra*	Atlantic coast of North Carolina and Florida	blubber		500.0													500 (ng/g d.w.) *	[90]
Mammalia	*Stenella* spp.	Atlantic coast of North Carolina and Florida	blubber		70.0													70.0 (ng/g d.w.) *	[90]
Mammalia	*Stenella coeruleoalba*	Macaronesian Region (Eastern North Atlantic)	muscles		86.2		698.7							513.9				1298.8 (ng/g w.w.)	[89]

Table 3. *Cont.*

Organisms	Species	Location in Wild	Samples	DMP	DEP	DiBP	DnBP	DMEP	DnPP	BBzP	DBEP	DCHP	DPHP	DEHP	DnOP	DiNP	DnNP	Sum of PAEs (unit)	Ref. Year
Mammalia	*Tursiops truncatus*	Atlantic coast of North Carolina and Florida	blubber		4800.0													4800.0 (ng/g d.w.) *	[90]
Mammalia	*Tursiops truncatus*	Macaronesian Region (Eastern North Atlantic)	muscles				413.0							783.0				1196.0 (ng/g w.w.)	[89]
Reptilia	*Caretta caretta*	Sicily, Campania, Sardinia (Mediterranean Sea)	blood	1.2	6.8	12.1	16.2							24.9	7.4			68.4 (ng/mL w.w.)	[91]
Reptilia	*Caretta caretta*	Sicily (Mediterranean Sea)	gonads	n.d.	n.d.		4520.8			173.7				325.5	104.2			5124.2 (ng/g w.w.)	[78]
Reptilia	*Caretta caretta*	Sicily (Mediterranean Sea)	liver	n.d.	n.d.		4046.9			2018.8				361.3	540.6			6967.5 (ng/g w.w.)	[78]
Reptilia	*Caretta caretta*	Sicily (Mediterranean Sea)	blubber	n.d.	n.d.		2411.0			360.0				4295.6	5481.2			12,547.8 (ng/g w.w.)	[78]
Reptilia	*Dermochelys coriacea*	Sicily (Mediterranean Sea)	gonads	n.d.	5718.0		12,166.7			12,532.6				5572.9	n.d.			35,990.2 (ng/g w.w.)	[78]
Reptilia	*Dermochelys coriacea*	Sicily (Mediterranean Sea)	liver	n.d.	3937.0		6055.6			16,014.5				1226.9	n.d.			27,233.9 (ng/g w.w.)	[78]
Reptilia	*Dermochelys coriacea*	Sicily (Mediterranean Sea)	muscles	n.d.	n.d.		2000.0			n.d.				n.d.	n.d.			2000.0 (ng/g w.w.)	[78]
zooplankton	size > 1000 µm	Marseille (Mediterranean Sea)	whole body	140.9	18.6	110.4	377.1			81.1				5586.7	262.9			6577.7 (ng/g d.w.)	[92]
zooplankton	size: 150–500 µm	Marseille (Mediterranean Sea)	whole body	52.0	33.7	73.9	183.6			63.2				6659.2	469.6			7535.4 (ng/g d.w.)	[92]
zooplankton	size: 500–1000 µm	Marseille (Mediterranean Sea)	whole body	63.9	20.2	46.1	130.2			71.5				2981.9	173.9			3487.7 (ng/g d.w.)	[92]

Values are calculated as a mean when available in the work, alternatively, values have been reported as median (*). Where possible, the units of measurement, indicated in brackets next to the sum of the phthalates, were converted to ng/g, followed by wet weight (w.w.) or dry weight (d.w.); n.d. = not detected.

Considering two tissue types from three fish species, the DiNP showed greater values in the gills than the muscles, with a higher average value recorded in the gills of *M. barbatus* (1491 ng/g d.w.). Similarly, the same sample shows a higher mean concentration value of DMP (649 ng/g d.w.) than the corresponding muscle sample, the other samples of the same work, and all samples of other organisms analysed in Table 3 (thirty-three fish, four bivalves, two crustaceans, one gastropod, one mammal, seven sea turtles, and three different zooplankton samples). High DEP values were found in fat samples of *L. albirostris* (13,800 ng/g d.w.) and *T. truncatus* (4800 ng/g d.w.) [90]. However, in the latter, the concentration is expressed in dry weight, despite the low water content in the fat samples (about 10%) [93] and the levels would be comparable to those found in the gonads (5718 ng/g w.w.) and liver (3937 ng/g w.w.) of sea turtles *D. coriacea* stranded in the coast of Sicily (Mediterranean Sea) [78]. High DEP values, although a smaller order of magnitude than those discovered in *D. coriacea*, were also observed in the soft tissues of the bivalve *A. noae* (540.0 ng/g w.w.) and in some holothurian species (490 ng/gw.w.) in the marine-protected area of Cabrera (Balearic Sea) [69], indicating that these animals may be considered good bioindicators of that substance.

Regarding the DiBP, the highest levels were found by Zhang et al. (2021) in China in the muscles of fish *C. striata* (1938 ng/g d.w.), *S. ocellatus* (1822 ng/g d.w.), and *T. ovatus* (1791 ng/g d.w.). For the DnBP, the highest levels were detected in sea turtles *D. coriacea* and *C. caretta* and, considering the different analysed tissues, the gonads showed greater contamination (12,166.7 ng/g w.w. in *D. coriaceous* and 4520.8 in *C. caretta*) [78]. DMEP, DnPP, DBEP, DCHP, and DPHP are analysed in one work reported in Table 3 [81] and the concentration values are relatively lower than the other investigated phthalates in the same work and, in general, in the table. The highest concentrations of BBzP are related to *D. coriacea* (16,014.5 ng/g w.w. in the liver and 12,532.6 ng/g w.w. in the gonads) [78]. Except for the work of Page-Karjian et al. (2020), which investigates only the DEP as phthalate, in all the research illustrated in Table 3, the DEHP has been studied. Significant levels of DEHP were observed in ascidians *H. momus* (9095.0 and 5556.0 ng/g d.w.) and *M. exasperatus* (4988.0 and 4851.0 ng/g d.w.) [84] in mussels (8355.6 ng/g d.w.) [86] and in zooplankton (size class: 150–500 µm and size > 1000 µm, 6659.2 and 5586.7 ng/g d.w., respectively) [92].

However, considering the high water content of the latter organisms (for example, zooplankton consists of 90% water [94]), the concentration values in wet weight would be lower. In this context, the highest average subsequent values are for the gonads of *D. coriacea* (5572.9 ng/g w.w.) and the fat of *C. caretta* (4295.6 ng/g w.w.) [78], or the soft tissues of bivalve *A. noae* (2580.0 ng/g w.w.) [69].

Regarding Actinopterygii, the highest levels of DEHP were detected in the muscles of *C. striata* (2981.9 ng/g d.w.) [81], whereas among mammals, the highest values were recorded in *T. truncatus* muscles (783.0 ng/g w.w.) [89]. Focusing on the DnOP, the greater value present in Table 3 concerns *C. caretta* fat (5481.2 ng/g w.w.) compared to other tissues, probably due to the high lipophilicity of this substance [78], followed by the gills of *M. barbatus* (1061.0 ng/g d.w.), which may be related to its physiological function [80]; instead, in the only work investigating DnNP, the highest value was observed for *L. japonicus* (8.2 ng/g d.w.) [81].

Considerable attention should be paid to the ratio of wet weight, dry weight, or lipid weight basis. In fact, considering the phthalates detected in the tissues of sea turtles, the maximum levels recorded for DEP, DnBP, BBzP, DEHP, and DEHT are among the highest in Table 3, probably due to the massive ingestion of plastic material by these sentinel organisms.

In the same work, differences in concentration for the same phthalate between different analysed organisms could depend on various states of environmental contamination of chosen sites within a large sampling area [82]. Sampling areas, that are more protected or further away from sources of pollution, are less prone to be contaminated as reported for DBP and DEHP in Ascidiacea collected in marine reserves [84]: these areas did not

differ from blanks with concentrations of three orders of magnitude lower than other less safeguarded sites. However, phthalates contamination is not always low in marine-protected areas (MPAs): it has been reported that, in the MPA of Cabrera (Balearic Sea), high levels of DMP, DEP, and DEHP have been recorded [69]. These PAEs differed between several species, highlighting that, within the same area, the ecological characteristics and feeding strategies play central roles in determining the degree of accumulation of phthalates in various organisms.

With regard to the variability of the concentration illustrated in the different works, seasonality plays an important role as observed by several authors, in particular in significant differences between the levels of concentration of the same phthalate in the same species [81,86,95].

For a correct assessment of the levels of the contamination of organisms and indirectly of the environment, it is important to consider the same environment, the same species, and possibly different tissues of the same organism. This would reduce the variability of the determination of substances linked to any instrumental or operator errors to differences between extraction and/or analytical processes, and the influence of environmental physicochemical parameters on the accumulation capacity of organisms, etc.

Moreover, although the physiological characteristics of the organisms and physicochemical parameters of the environment determine the degree of contamination of the exposed organisms and their biodistribution, appropriate tissues are not always considered in the various studies.

Biodistribution studies are generally associated with pharmacokinetic approaches in which the rate and extent of distribution of the drug after its application are evaluated [96]. Similarly, in the environmental sciences, the biodistribution of a pollutant can be defined as the study of the concentration levels of a given substance in a given organism in different tissues or biological elements (cells, tissues, organs), in other words, the fate of the substance within an organism and its distribution profile in different tissues [32]. In this field, the degree of affinity or localisation tendency of a substance within a biological system can be defined as organotropism [97].

Generally, once the chemical has entered the body through the vascular system, it is distributed in different tissues based on its physicochemical property to its ability to penetrate barriers [98].

This distribution is the result of a dynamic and complex process that presents differences both intraspecific and interspecific. There are therefore several variables that affect the biological system (the species, sex, physiological mechanisms of transport of the substance, the rate of distribution of the substance, the nature and mass of the tissue, the district pH, the degree of permeability of cell membranes, and more generally, the metabolism and rate of excretion) [32].

A proper assessment of the levels of PAEs contamination should consider all the environmental matrices. Additionally, a correlation between the obtained results and the above factors should be made.

5. Environmental Perspective

It is widely recognised that anthropogenic activities can cause environmental pollution, affecting ecosystems and their member organisms. The aquatic environment, especially the marine ecosystem, is strongly influenced by this contamination, since in many cases, it represents the final destination of all waste [99]. This environment is a precious heritage that must be protected, safeguarded and, where possible, restored to maintain biodiversity and preserve the vitality of clean, healthy, and productive seas and oceans. Unfortunately, today, there is a growing concern due to the ubiquitous spread of potentially dangerous chemicals that can be bioconcentrated and/or biomagnified. Among the emerging pollutants, phthalates have long been of particular interest due to the environmental impact of plastics on the planet. PAEs can lead to numerous chronic and fatal diseases and have been detected in all environments. On the other hand, PAEs as endocrine-disrupting chemicals

(EDCs) [100] can alter, in different ways, the normal hormonal activity of the biological system and therefore affect its physiological homeostasis, causing the onset of different diseases that can lead to death.

Contamination of the trophic network negatively affects the health of its components, including humans. The effects depend on the biodistribution of the bioactive substance and the complex integrated system of functions involved. As result, it is necessary to assess the extent of pollution through biomonitoring studies based on appropriate bioindicators that provide useful information for the determination of environmental stress. Noteworthy, several bacterial strains, fungi, plants, and algae have been reported to both biosynthesise and degrade phthalates [101,102]. Therefore, it is essential to thoroughly investigate the biological activities of the organism to evaluate possible applications of environmental bioremediation. Considering the characteristics of some promising poorly studied bioindicators such as algae, indicative studies should be undertaken to determine whether they can be used to monitor phthalate pollution. For this purpose, by accurately assessing their bioaccumulation capacities, these organisms could be considered for environmental bioremediation studies aimed to minimise and counter the environmental impact related to PAEs.

In this context, the comparative analysis of the main PAEs and their metabolites should always be carried out because when the first compound enters the organism, it could easily metabolise, leading to the formation of high amounts of toxic products. Given the extensive metabolism of PAEs to monoesters, precursors and intermediates should be considered in order to avoid an underestimation of the incidence of phthalate contamination and its derivatives.

Recent research have focused on the analysis of phthalate products, some of which are called pollution markers for their precursors because they are generally more present. However, considering that metabolite levels may be lower than those of their precursor, an analytical method considering both types of compounds should be used [82]. At the same time, extraction and analysis methods should be improved to avoid underestimation due to low analyte recovery or a high limit of detection.

Several other biomonitoring work should be carried out to better understand the incidence of these substances, which is likely to increase. This work should therefore consider both the main phthalates (most widely used and therefore released into the environment), their alternatives, and their metabolites. Whitin this framework, further toxicity analyses should be carried out to fully determine the effect of these substances, which are particularly detrimental to the most sensitive individuals.

Similarly, alternatives to phthalates should be studied carefully to understand all the negative aspects of exposure.

The enrichment of knowledge and the updating of toxicity limits would lead to an increased awareness by nations and the adoption of further control measures.

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