NET4mPLASTIC Project

Work Package 3.4

Deliverable 3.4.1

GUIDELINES AND INDICATORS EDITING FOR PROPER CONSUMPTION OF MUSSEL AND SHELLFISH TO PREVENT TOXICITY AND HUMAN HEALTH RISKS

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SUMMARY

This document refers to Deliverable 3.4.1, which is related to edit “Guidelines and Indicators for proper consumption of mussels and shellfish to prevent toxicity and human health risks”. National and International laws / directives as well as scientific papers / reviews were analyzed in order to evaluate both the exposure of the environment and the organisms to micro and macro plastics, focusing on sea water and marine biota, and the legislative actions currently applied by different countries.

The main aims of the Deliverable 3.4.1 were:

- Evaluation of the potential toxic effects of plastics / additives and related contaminants on organisms, including human.
- Evaluation of the political and legislative actions concerning plastics / microplastics and related contaminants.
- Evaluation of the laws and directives concerning the sea water pollution.
- Evaluation of the official regulation of sea water contaminants (Polycyclic Aromatic Hydrocarbons [PAHs], Polychlorinated Biphenyls [PCBs], dioxins and heavy metals) and their occurrence in shellfish.
- Estimation of microplastics concentrations in the marine biota, focusing on mussels.

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1. Introduction

Plastic is currently identified as the geological indicator of the era dominated by human, termed "Anthropocene", due to its spread both as component of human life as well as waste and debris in the environment [Zalasiewicz J. et al.; 2016]. Moreover, as consequence of the non-degradable nature, plastic items are accumulated in landfills, rivers and oceans assuming the role of emerging environmental pollutants [Lam C. S. et al.; 2018]. Notwithstanding the social benefits and their involvement in several sectors of human life (e.g. health, agricultural, packaging, construction/building and textiles), a considerable portion of the produced plastic each year persists in the marine environment [Wright L. S. et al.; 2017 – Rodrigues M. O. et al.; 2019]. The global analysis of the plastics production and waste management in 2015 exhibited a dangerous situation, where the 79% of plastics was accumulated in landfills or in natural environment, the 12% was incinerated and only the 9% was recycled [Geyer R. et al.; 2017]. However, despite the continuous increasing plastic demand and consequence production, several countries are introducing policies to promote recovery and recycling economies, reducing the plastic leakage in the environment. In 2018, 359 million of plastic tons were manufactured in the world, for which Asia contributed with about half of the total production (51%), while Europe was responsible of 61.8 (nearly 17%) million tons of plastic items [PlasticsEurope; 2019]. Considering the 29.1 million tons of plastic post-consumer waste collected in Europe during the year 2018, the 32.5% was recycled, the 42.6% was incinerated and the remaining 24.9% was accumulated in landfills ("Conversion Market & Strategy GmbH" evaluation) [PlasticsEurope; 2019]. In this context, seas and oceans may appear as receptacles of all environmental pollutants since they collect the waste voluntarily trashed by human, transported by the weather (i.e. wind, rain) and/or by the river flows within their waters [Battulga B. et al.; 2019 - Montuori P. et al.; 2016]. Recently, the oceans will be estimated to contain more plastics than fish by the year 2050 [World Economic Forum; 2016].

The accumulated plastic litter requires long time to totally degrade and furthermore, plastic items are fragmented into smaller particles following photo-oxidation, ultraviolet (UV) exposition, mechanical stress (e.g. friction by waves and wind or washing of synthetic clothes) and bacteria action that break the chemical bonds of the polymer chains [Andardy A. L.; 2011 – Galafassi S. et al.; 2019]. The Microplastics [MPs] obtained by the abiotic and biotic fragmentation (secondary MPs) together to the primary MPs (voluntarily produced by human, such as MPs contained in personal care products) could be ingested by fishes, molluscs, birds and reptiles potentially exerting physical damage and toxic effects on them [Conkle J. L. et al.; 2018 - Koelmans A. A. et al; 2016]. Ingested Polystyrene [PS] MPs accumulated in gut tissue and altered the expression of genes, up-regulating the transcription of genes involved in the mussel development and the immune response (e.g. chitin synthase and myticin C, respectively) and, inhibiting the lysosomal enzymes expression in the Mediterranean mussel *Mytilus galloprovincialis* [Capolupo M. et al.; 2018]. Moreover, trophic transfer from preys to predators of ingested particles may shift the plastic toxicity along the food chain, till the human [Zhang S. et al.; 2019]. On the other hand, large plastic items can entangle marine organisms, such as turtles and dolphins, causing their suffocation and death [Byard R. W. et al.; 2020].

The potential toxicity of plastics is enhanced both by the components added during the plastic production and, by the adsorption of environmental pollutants. Solvents, diluents, catalysts, initiators, antioxidants,

plasticizers, elastomers and stabilizers are added to plastic monomers during the polymerization reaction in order to improve the plastic yield and properties [Hahladakis J. N. et al.; 2018]. These additives, together to the remaining monomers and oligomers, are not bound to the plastic polymer matrix and could migrate into the environment or in the substances in contact with plastic, such as food [Qian S. et al.; 2018]. Furthermore, metals and Persistent Organic Pollutants [POPs] (e.g. Polycyclic Aromatic Hydrocarbons [PAHs] and Polychlorinated Biphenyls [PCBs]) can be adsorbed on the plastic surface [Ashton K. et al.; 2010 – Li J. et al.; 2018]. Hence, plastics could be vehicles of hazardous compounds, as well as pathogenic or antibiotic resistant bacteria that colonized the plastic surface carrying them from lands into the water systems and thus to seas and oceans [Galafassi S. et al.; 2019].

This deliverable aims to provide a comprehensive review of the toxicity of plastics, related additives and environmental pollutants, focusing on sea water contaminants, and a brief overview of the currently legislations applied by different countries in order to reduce the plastic pollution. Moreover, guidelines on proper mussels and shellfish consumption will be suggested to prevent human health risks.

2. Impact of Plastics on Human Health

2.1. Toxicity of Plastics

Plastic is a large family of different materials produced by different sources (e.g. crude oil, sugar cane, vegetable oils, salt) and with specific characteristics and uses [PlasticsEurope; 2018]. The family of plastics can be divided into two major categories: i) thermoplastics and ii) thermosets. Thermoplastics such as Polyethylene [PE], Polypropylene [PP] and Polyvinyl Chloride [PVC], can be reheated and frozen several times. On the other hand, thermosets change the chemical structure after heating, creating a 3D network, hence they cannot be subsequently melted (e.g. Polyurethane [PUR], epoxy resins, silicone) [PlasticsEurope; 2019]. Altogether, plastics can be classified in accordance to the monomer composition and hazard classification: Polyacrylonitriles [PANs], PU, PVC, Epoxy Resins and Styrenic Copolymers are classified as the most dangerous plastics since they are made of carcinogenic and/or mutagenic monomers (category 1A or 1B) based on the Annex VI of the European Parliament and Council Regulation (EC) No 1272/2008 [Lithner D. et al.; 2011 - European Parliament and Council; 2008].
Considering the plastic demand in Europe in 2018, the most produced polymers are PP (19.3%) and PE (between 17.5 and 12.2%), followed by PVC (10%), PUR, Polyethylene Terephthalate [PET] and PS (about 7%) that are used especially in the packaging and building sectors (Table 1) [PlasticsEurope; 2019].

Table 1: Summary of plastics demand in Europe and the related hazard levels based on Lithner D. et al. classification.

Polymer	Plastic demand (%)	Main Sector	Hazard Class Monomer	Hazard Level Polymer
Polypropylene (PP)	19.3%	Packaging	Flammable	I
Polyethylene-Low Density (LD-PE)	17.5%	Packaging	STOT-SE	II
Polyethylene-High Density (HD-PE)	12.2%	Packaging	STOT-SE	II
Polyvinyl Chloride (PVC)	10%	Building, Construction	Carc. 1A	V
Polyurethane (PUR)	7.9%	Building, Construction	Carc. 1B and Muta. 1B	V
Polyethylene Terephthalate (PET)	7.7%	Packaging	Acute toxicity (oral, dermal, inhalation); Eye and skin irritation	II
Polystyrene (PS)	6.4%	Packaging	Acute toxicity (oral, dermal, inhalation); Eye and skin irritation	II

Carc. = Carcinogenicity; Muta. = Germ cell Mutagenicity; STOT-SE = Specific Target Organ Toxicity -Single Exposure. Hazard Levels (from I to V) based on environment and health hazards. V = most hazardous [PlasticsEurope; 2019 – Lithner D. et al.; 2011].

Toxicity of plastics on human health is still a matter of debate, since the absence of studies on humans *in vivo* and few evidences of plastic effects on human cell lines. Although, the hydrophobic polymer chains of plastics could potentially fit within the lipid bilayer of cell membrane influencing its biological functions

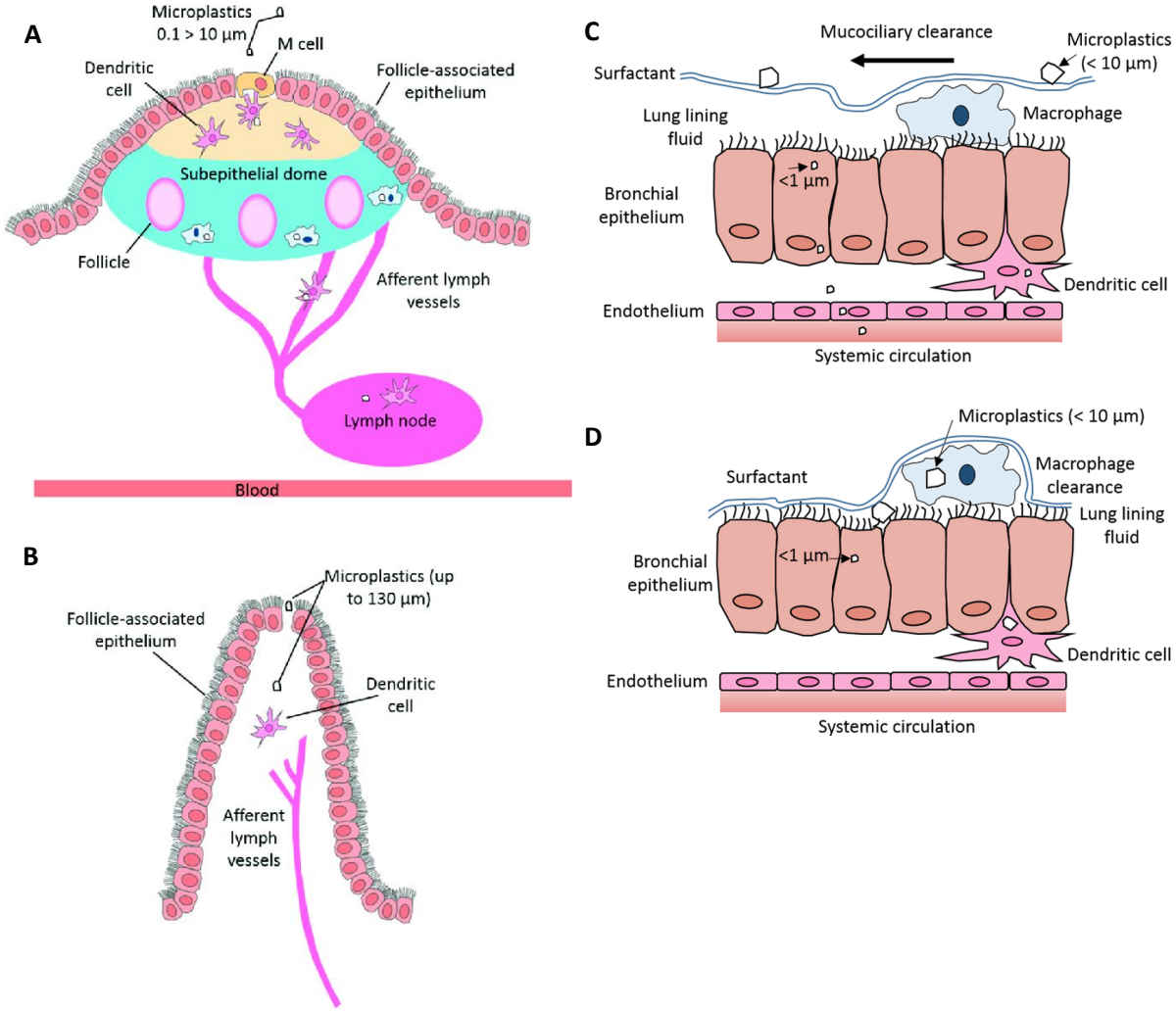
and leading the cell death [Holloczki O. et al.; 2020]. Toxicological and pathological effects of plastics have been well documented especially in marine organisms, such as fishes and molluscs. PS and PE particles were the more used in these studies, moreover, smaller debris have been generally associated to plastic toxicity rather than the particles of higher dimension [Cheryl Q. Y. Y. et al.; 2020]. However, the laboratory experiments were performed using virgin polymers that allowed to understand the behaviour of the polymers [Rodrigues M. O. et al.; 2019] but lacking the influence of the substances added during the plastic production (i.e. monomers and additives). PS MPs and NPs accumulated in gill and liver of adult and larvae Zebrafish, causing changes in metabolomic profiles, oxidative-stress response and alteration in feeding, movement and reproduction [Cheryl Q. Y. Y. et al.; 2020]. Cation PS NPs induced a firstly inflammatory response (e.g. extracellular reactive oxygen species [ROS] and nitric oxide production) and activated apoptotic process in a dose-dependent and time-dependent manner in PS-NH_2 NPs treated marine bivalve *Mytilus galloprovincialis* [Canesi L. et al.; 2015]. Liver, kidney and gut accumulation of PS MPs was observed in mice treated with a mixture of plastic particles and water. Deposits of PS MPs induced the expression of oxidative stress enzymes (i.e. Superoxide Dismutase 1 [SOD1] and Glutathione Peroxidase 1 [GSH-Px1]) and altered the lipid metabolism (i.e. low levels of total Cholesterol and triglycerides) in liver [Deng Y. et al; 2017].

Concerning the potential toxicity of plastic on human, ROS production and pro-inflammatory responses were observed in several studies performed on human cell lines [Cheryl Q. Y. Y. et al.; 2020]. Treatment with PE or PS MPs induced ROS production and cytotoxicity in T98G and HeLa cells (human glioblastoma cell line and cervical adenocarcinoma cell line, respectively) [Schirinzi G. F. et al.; 2017]. Production of cytokines (i.e. IL-6 and TNF-α) and ROS and release of histamine were observed respectively in Peripheral Blood Mononuclear Cells [PBMCs] and in Human Mast Cell line 1 [HMC-1], following the treatment with PP MPs [Hwang et al.; 2019]. On the other hand, some studies showed the absence of plastic toxicity on human cell lines. For example, no significant toxic effects, except at very high dosage, were evaluated in PS MPs treated- Caco-2 cells and THP-1 monocytic line [Stock V. et al.; 2019].

Moreover, the uptake and the fate of plastics within the human body have been hypothesized based on the results obtained on other organisms and on cell lines. MPs could reach the human Gastrointestinal Tract [GIT] or lung after ingestion or inhalation, where they could accumulate causing acute and chronic inflammation, enter the circulation or be excreted. Uptake of inhaled and ingested MPs occurs via endocytosis by the respiratory epithelium and the M cells of the Peyer's patches in the GIT, other than the diffusion of particles among the gaps of the villus tips (Persoption route within the GIT) [Wright L. S. et al.; 2017]. Accumulation of plastic debris in the GIT could alter the gut microbiome leading to disorders in other organs, such as liver and kidney [Cheryl Q. Y. Y. et al.; 2020]. However, the higher hydrophobic particles may not be accumulated in the lung, but they could be subjected to mucociliary clearance and transported to the GIT increasing its plastic exposure [Wright L. S. et al.; 2017]. Following the uptake, particles could be carried by macrophages and dendritic cells toward lymph nodes or transported by Human Serum Albumin [HSA] (i.e. proteins corona: particles surrounded by HSAs) within the systemic circulation reaching secondary organ targets, such as liver, kidney, spleen, heart and brain [Geiser M. et al.; 2014 - Kihara S. et al.; 2019]. Finally, elimination of ingested plastics was observed in human. A median of 20 particles and fibres of seven different plastic types per 10 g of sample were evaluated in the stools of eight healthy volunteers [Schwabl P. et al.; 2019].

Potential pathways of plastic particles uptake in the gastrointestinal tract and lung are shown in Figure 1 [Wright L. S. et al.; 2017].

Figure 1: Schematic representation of predicted MPs uptake in the gastrointestinal tract [GIT] (A-B) and lung (C-D). A) MPs uptake from the GIT lumen via endocytosis by the M cells of the Peyer's patches and the following transport



to the mucosal lymphoid tissues; B) MPs uptake from the GIT lumen via paracellular persoption and the following transport by the dendritic cells to the lymphatic vessels and veins; C) MPs uptake across the lung epithelium for MPs < 1 µm, while particles > 1 µm are subjected to mucociliary clearance; D) Potential MPs penetration within the lung epithelium through diffusion or active cellular uptake overcoming the clearance mechanisms (i.e. macrophages and lung lining fluid (composed by surfactant and mucus)) [Ruge C. A. et al.; 2013 – Mowat A. M.I.; 2003].

Evaluation of the risk of plastic materials on human health are evidenced in studies regarding the occupational exposure within the synthetic textile, the flock and the PVC industries, where workers may be exposed to higher plastic concentrations than the environmental levels [Prata J. C.; 2018]. Nylon, polyester, polyurethane, polyolefin and acrylic are the mainly component of the synthetic fibre dusts that have been related to respiratory clinical symptoms, such as the bronchopulmonary diseases (e.g. asthma and allergic alveolitis) observed in synthetic textile workers. Interstitial fibrosis and granulomatous lesions

containing foreign body were identified within the lung biopsies of synthetic fibre dusts exposed-Guinea pigs reproducing the respiratory disease [Pimentel J. C. et al.; 1975]. Moreover, a meta-analysis of epidemiological studies suggested different cancer developments after chronic exposure to persistent synthetic fibres in relation to the type of fibre and the kind of job, such as cancers in the bladder or in the nasal cavity observed among weavers exposed to textile dust [Mastrangelo G. et al.; 2002]. Nylon, polyester, PE and PP composed the flock fibres used to manufacture velvet-like and fleeced clothes through their application on adhesive-coated materials. The cutting process for the generation of useful 0.2-0.5 mm flock causes smaller particles that may be responsible of the interstitial lung disease (flock's disease) inducing cough, dyspnea, interstitial pneumonitis and reduced lung capacity [Eschenbacher W. L. et al.; 1999]. Long exposure to PVC dust was associated to accumulation of macrophages and foreign body giant cells, fibrotic changes of the alveolar wall and dyspnea in workers of the PVC industries [Antti-Poika M. et al.; 1986 – Studnicka M. J. et al.; 1995]. Furthermore, PVC and vinyl chloride exposition may be responsible of an increased risk in lung cancer in exposed workers as evaluated in an Italian cohort of PVC plant workers involved in different steps of the industrial process (i.e. vinyl chloride production, PVC resin production and PVC compounding) [Mastrangelo G. et al.; 2003].

2.2. Toxicity of Additives

Other than the polymer chains and the eventual monomers and oligomers resulting from the polymer reaction, plastics contain additives that differ among the several types of materials (Table 2) [Rodrigues M.O. et al.; 2019]. These chemical compounds improve the production performance (e.g. extrusion, vacuum/blow moulding), the ageing and the functional properties of the polymer [Hahladakis J. N. et al.; 2018]. For example, PVC requires heat stabilizers and plasticizers to prevent its degradation and ensure its flexibility; PP needs antioxidants and UV-stabilizers to prevent its oxidation while antistatic agents and flame retardants allow its use as component of electronics, construction and transportation applications [Zweifel H. et al.; 2001]. PVC requires the major number of additives, followed by PE, PP and PS [Rodrigues M. O. et al.; 2019].

The most commonly used additives are: plasticizers, flame retardants, antioxidants, acid scavengers, light- heat- and thermal stabilizers, lubricants, pigments, antistatic agents and slip compounds. They can be grouped into 4 categories [Hahladakis J. N. et al.; 2018]:

1. Functional additives = stabilizers, antistatic agents, flame retardants, plasticizers, lubricants, slip agents, curing agents, foaming agents, biocides, etc.;
2. Colorants = pigments, soluble azocolorants, etc.
3. Fillers = mica, talc, kaolin, clay, Calcium Carbonate, Barium Sulphate;
4. Reinforcements = glass fibres, carbon fibres, etc.

Table 2: Summary of the most commonly used additives in plastic material based on Hahladakis J. N. et al. classification.

Category	Typical amount range (% w/w)	Additives	Additional comments
Plasticizers	10-70	Short/Medium/Long Chain Chlorinated Paraffins [SCCP/MCCP/LCCP]; Diisoheptylphthalate [DIHP]; Benzyl Butyl Phthalate [BBP]; Bis(2-ethylhexyl)phthalate [DEHP]; Bis(2-methoxyethyl)phthalate [DMEP]; Dibutyl Phthalate [DBP]; Dipentyl Phthalate [DPP]; Di(2-ethylhexyl)adipate [DEHA]; Di-octyladipate [DOA]; Diethyl Phthalate [DEP]; Diisobutylphthalate [DiBP]; Tris(2-chloroethyl)phosphate [TCEP]; Dicyclohexyl Phthalate]DCHP]; Butyl Benzyl Phthalate [BBP]; Diheptyl Adipate [DHA]; Heptyl Adipate [HAD]; heptyl Octyl Adipate [HOA].	About 80% is used in PVC and 20% in cellulose plastic.
Flame retardants	3-25 (for brominated)	SSCP/MCCP/LCCP; Boric Acid; Brominated flame retardants with Antimony as synergist (e.g. Polybrominated Diphenyl Ethers [PBDE], Decabromodiphenylethane, Tetrabromobisphenol A [TBBPA]); Phosphorus flame retardants (e.g. Tris(2-chloroethyl) phosphate [TCEP], Tris(2-chlorisopropyl) phosphate [TCPP]).	Three groups: i) Organic non-reactive (e.g. Phosphate Esters, Halogenated Phosphate Esters, Halogenated Hydrocarbons); ii) Inorganic non-reactive (e.g. Antimony Oxide, Aluminium Oxide trihydrate, Zinc Borate, Ammonium Orthophosphate, Ammonium Sulfamate); iii) Reactive (Bromine and/or Phosphorus containing polyols, Halogenated Phenols, Tetrachlorophthalic Anhydride, Phosphonate Esters, Dibromoneopentyl Alcohol).
	0.7-3	Hexabromocyclohexane [HBCDD].	

Stabilizers, Antioxidant and UV-stabilizers	0.05-3	Bisphenol A [BPA]; Cadmium and Lead compounds; Nolylphenol compounds; Octylphenol; 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione [TGIC] / 1,3,5-tris[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H,3H,5H)-trione [b-TGIC]; Butylated Hydroxytoluene [BHT]; 2- and 3-t-butyl-4-hydroxyanisole [BHA]; Tetraskimethylene-(3,5-di-t-butyl-4-hydroxycinnamate) methane [Irganox 1010] and bisphenolics (e.g. Cyanox 2246 and 425); Tris-nonyl-phenyl phosphate [TNPP]; Tris(2,4-di-ter-t-butylphenyl)phosphite [Irgafos 168].	The amount depends on the chemical structure of the additives and of the plastic polymer. Phenolic antioxidants are used in low amounts and phosphites in high. Lowest amounts in polyolefins (LLD-PE, HD-PE), higher in HIPS and Acrylonitrile-butadiene-styrene [ABS].
Heat stabilizers	0.5-3	Cadmium and Lead compounds; Nonylphenol (Barium and Calcium salts).	Mainly used in PVC. Based on Pb, Sn, Ba, Cd and Zn compounds. Pb is the most efficient and it is used in lower amounts.
Slip agents	0.1-3	Fatty acid amides (primary erucamide and oleamide), fatty acid esters, metallic stearates (e.g. Zinc stearate) and waxes.	The amounts are dependent on the chemical structure of the slip agent and the plastic polymer type.
Lubricants (internal and external)	0.1-3	-	-
Anti-statics	0.1-1	-	Most types are hydrophilic with the potential to migrate to water.
Curing agents	0.1-2	4,4'-Diaminodiphenylmethane [MDA]; 2,2'-dichloro-4,4'-methylenedianiline [MOCA]; Formaldehyde reaction products with aniline; Hydrazine; 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinanen-2,4,6-trione [TGIC] / 1,3,5-tris[(2S and 2R)-2,3-epoxypropyl]1,3,5-triazine-2,4,6-(1,3H,5H)-trione [b-TGIC].	Peroxides and other crosslinkers, catalysts, accelerators.
Blowing agents	Depends on the density of the foam and the potential gas production of the agent.	-	Azodicarbonamide; Benzene Disulphonyl Hydrazide [BSH]; Pentane; CO_2.

Biocides	0.001-1	Arsenic compounds; Organic Tin compounds; Triclosan.	Soft PVC and foamed polyurethanes are the major consumers of biocides. They vary in chemical structures and include chlorinated nitrogensulphur heterocycles and compounds based on Sn, Hg, As, Cu and Sb (e.g. Tributyltin and 10,10'-oxybysphenoarsine).
Colorants Soluble (e.g. Azocolorants)	0.25-5	-	They migrate easily and are used in highly transparent plastics. They are expensive, with limited light and heat resistance. Mostly used in PS, PMMA and cellulose plastics to give a bright transparent colour.
Organic pigments	0.001-2.5	Cobalt (II) Diacetate	They are insoluble with low migration tendency.
Inorganic pigments	0.01-10	Cadmium compounds; Chromium compounds; Lead compounds.	e.g. Zinc Sulphide, Zinc Oxide, Iron Oxide, Cadmium-Manganese based, Chromium based, ultramarine and Titanium Dioxide.
Special effects	Varies with the effect and substance in question	-	Aluminium and Copper powder, Lead Carbonate or Bismuthoxichloride and substances with fluorescence might migrate, the former not.
Fillers	Up to 50	-	Calcium Carbonate, talk, clay, Zinc Oxide, glimmer, metal powder, asbest, Barium Sulphate, glass microspheres, silicous earth.
Reinforcements	15-30	-	Glass fibers, carbon fibers, aramide fibers. 15-30% is for glass only due to is high density.

"-" = not information available [Hahladakis J. N. et al.; 2018]

Several additives are recognized as medium-high hazardous compounds, including phthalate (plasticizer)

and Lead [Pb] (stabilizer) (Table 3). Moreover, since they are of low molecular weight and not bound to the polymer matrix, they are often leached by the plastic materials [Lithner D. et al.; 2011]. Phthalates, Bisphenol A [BPA] and Bisphenol S [BPS] are plasticizers identified as "Endocrine Disrupting Chemicals" [EDC], since they interfere with the endocrine system, as evidenced by the reduction of spermatogenesis in the offspring and the alteration of the expression of estrogen receptors $\alpha/\beta/\gamma$ in mice treated with Dibutyl Phthalate [DBP] and BPA, respectively [Palanza P.; 2017 – Yuan B. et al.; 2017 - Kundakovic M. et al.; 2013].

Benzene, Cadmium and 1,3-butadiene are recognized as cancerogenic compounds by the International Research on Cancer (IARC) [IARC; 2020]. Their exposure is associated to the development of leukemia (i.e. benzene and 1,3-butadiene) and solid cancers such as lung, prostate and kidney cancers (i.e. Cadmium; recently, benzene has been related to lung cancer) [Arnold S. M. et al.; 2013 – Warden H. et al.; 2018 – Huy L. et al.; 2018]. While, other additives cause less severe effects on environment and human health, such as ethanol and acetic acid, whose toxicities are only associated to the flammable property of the compounds [Lithner D. et al.; 2018]. Furthermore, the presence of the additives may be enough to carry out their hazardous effects, however other parameters may influence the level of toxicity. Concentration of the compounds, time of exposure and the formulation, hence the interactions among the compounds, should be considered for a comprehensive evaluation of the hazard. For example, the combination of benzene and toluene did not alter the kinetic of benzene in the blood of treated human volunteers and animal, but a reduction in both metabolites was evaluated as consequence of their co-exposure probably related to their competition for the same metabolic enzymes. Decrease of the benzene metabolites (e.g. benzoquinone) protected from the formation of chromosomal aberrations in combined benzene and toluene treated mice [Medinsky M. A. et al.; 1994].

Table 3: Summary of toxicity and hazard level associated to monomers and additives based on European regulation hazard classes from Lithner D. et al. and European Parliament and Council 2008.

Hazard Level	Toxicity	Monomers	Additives	Associated polymers
Very low	Flammable liquid and vapour	Styrene	Chlorobenzene; Acetic acid	PS, EPS, HIPS, HD-PE, PA6
	Highly flammable liquid and vapour		n-hexane; Heptane; Toluene; Cyclohexane; Isoctane; Di-tert-butyl peroxide; Benzene; Ethanol; Isopentane; Ethylbenzene; Pentane	PP, HD-PE, HIPS, LLD-PE, LD-PE, PET, EPS
	Extremely flammable gas	Propylene; Ethylene; 1-butene; Vynil chloride	Iso-butene; 1,3-butadiene	PP, HD-PE, LLD-PE, LD-PE, PVC, HIPS
Low	May cause respiratory irritation	ε-caprolactam; Hexamethylenediamine	p-benzoquinone; Dipotassium peroxidisulphate; Diammonium perxodisulphate	PA6, PA6.6, PA6.10, PS, PVC

	Skin corrosion/irritation	Styrene; ε-caprolactam	n-hexane; Heptane; Toluene; Cyclohexane; Isooctane; Benzene; p-benzoquinone; Di-potassium peroxodisulphate; Diammonium peroxodisulphate	PS, EPS, HIPS, PA6, PP HD-PE, LLD-PE, LD-PE, PVC
	Serious eye damage/eye irritation	Styrene; ε-caprolactam; Adipic acid	Dibenzoyl peroxide; Benzene; p-benzoquinone; Di-potassium peroxodisulphate; Diammonium peroxodisulphate	PS, EPS, HIPS, PA6.6, PA6, LD-PE, PVC
	Harmful if swallowed, inhaled and/or contact with skin	Ethylene glycol; ε-caprolactam; Hexamethylendiamine; Styrene	2,2'-dimethyl-2,2'-azodipropionitrile; Dipotassium peroxodisulphate; Diammonium peroxodisulphate; Hydrogen peroxide; Heat stabilizers (Lead); Chlorobenzene; Ethylbenzene	PET, PA6, PA6.6, PA6.10, PS, EPS, HIPS, HD-PE, LD-PE, PVC
	Drowziness or dizziness	Ethylene	n-hexane; Heptane; Toluene; Cyclohexane; Isooctane; Isopentane; Pentane	PP, HD-PE, HIPS, LLD-PE, EPS
	Harmful to aquatic life with long-term effects		2,2'-dimethyl-2,2'-azodipropionitrile	LD-PE, PS, PVC
Medium	Ingestion, inhalation and/or dermal toxicity		Methanol; p-benzoquinone	PP, HD-PE, PS, LD-PE, PET, PA6.6, PA6.10
	Severe skin burns and eye damage	Hexamethylendiamine	Titanium tetrachloride; Acetic acid; Acetic acid; Phosphoric acid; Hydrogen peroxide	PA6, PA6.6, PA6.10, PVC, PP, HD-PE, LLD-PE, PA12
	Suspected of carcinogenicity		Fuels; Antimony trioxide	HD-PE, PET
	Suspected of damaging fertility		n-hexane; Heat stabilizers (Lead, Cadmium); Benzyl Butyl Phthalate	PP, HD-PE, PVC
	Suspected of damaging the unborn child		Toluene; Heat stabilizers (Cadmium)	HD-PE, HIPS, PVC
	Damage to organs through prolonged or repeated exposure		n-hexane; Toluene; Benzene; Heat stabilizers (Lead, Cadmium)	PP, HD-PE, HIPS, LD-PE, PVC
	May be fatal if ingested and inhaled		n-hexane; Heptane; Naphta; Toluene; Cyclohexane; Isooctane; Benzene; Isopentane; Pentane	PP, HD-PE, HIPS, LLD-PE, LD-PE, HD-PE, EPS

	Very toxic to aquatic life		Heptane; Cyclohexane; Isooctane; Zinc oxide; p-benzoquinone; Heat stabilizers (Lead, Zinc powder, Cadmium); Benxyl Butyl Phthalate	PP, HD-PE, LLD-PE, PS, PVC, PET
	Toxic to aquatic life with long-term effects		n-hexane; Chlorobenzene; Isopentane; Pentane	PP, HD-PE, LLD-PE, EPS
High	Fatal if inhaled		Heat stabilizers (Cadmium)	PVC
	Suspected of germ cell mutagenicity, Damage to organs through prolonged or repeated exposure		Methanol	PP, LD-PE, PET, PA6.6., PA6.10
	Very toxic to aquatic life with long-term effects		Heptane; Cyclohexane; Isooctane; Zinc oxide; p-benzoquinone; Heat stabilizers (Lead, Zinc powder, Cadmium); Benxyl Butyl Phthalate	PP, HD-PE, LLD-PE, PS, PVC, EPS, LD-PE, PET
	Allergic skin reaction		Dibenzoyl peroxide; Dipotassium peroxodisulphate; Diammonium peroxodisulphate	LD-PE, PS, EPS, PVC
	Respiratory difficulties/Asthma symptoms/Allergy		Dipotassium peroxodisulphate; diammonium peroxodisulphate	PS, PVC
Very high	Carcinogenicity	Vinyl chloride	Benzene; 1,3-butadiene; Naphtha; Heat stabilzers (Cadmium)	LD-PE, HIPS, PVC, PP
	Germ cell mutagenicity		Benzene; 1,3-butadiene; Naphtha	LD-PE, HIPS, PP
	Damage fertility and/or unborn child		Heat stabilizers (Lead); Benzyl Butyl Phthalate	PVC

EPS = PS expanded; HIPS = High Impacted PS; PA = Polyamide; PA6, PA6.6, PA6.10, PA12 = Polyamide 6, 6.6, 6.10, 12; PE = Polyethylene; LLD-PE = Linear Low Density-PE; LD-PE = Low Density PE; HD-PE = High Density-PE; PET = Polyethylene Terephthalate; PP = Polypropylene; PS = Polystyrene; PVC = Polyvinyl chloride [Lithner D. et al.; 2011 – Rodrigues M. O. et al.; 2019 – European Parliament and Council; 2008].

2.3. Toxicity of Plastic related to Environmental Pollutants

Plastics may adsorb environmental pollutants due to their hydrophobicity and large surface area concentrating the contaminants and increasing their potential toxicity. Heavy metals (e.g. Aluminium [Al], Copper [Cu], Mercury [Hg], Lead [Pb] and Zinc [Zn]) and Persistent Organic Pollutants [POPs] (e.g. Polychlorinated Biphenyls [PCBs], Polycyclic Aromatic hydrocarbons [PAHs] and pesticides such as, Dichlorobiphenyl Trichloroethane [DDT]) can be adsorbed by plastic items that act as vectors, transporting and protecting the contaminants, and increasing their bioavailability to the aquatic organisms [Rodrigues

J. P. et al.; 2019 – Rodrigues M. O. et al.; 2019]. For example, Hg is recognized by the World Health Organization [WHO] as one of the ten most dangerous chemicals to human, since its neurotoxicity and immunotoxicity [Biorklund G. et al.; 2017]. DNA damage and mortality were observed in Cu and Zn treated-*Alitta virens* worms [Watson G. J. et al.; 2018]. Since the hydrophobicity of POPs, they may accumulate in the fatty tissues, as observed in adults and embryos Zebrafish treated with benzo(a)pyrene (a PAH)-coated MPs [Batel A. et al.; 2018].

Several factors influence the adsorption of environmental pollutants on plastic surface concerning both plastic characteristics such as the age, the type and structure of the polymer, and environmental properties (e.g. water pH and salinity, temperature) [Gallo F. et al.; 2018]. For example, the larger the available plastic surface, the greater the contaminants potentially retained [Velzeboer I. et al.; 2014]. Moreover, the additives influence the dioxin-like compounds adsorption by the expanded PS [Chen Q. et al.; 2019]. Evaluation of the sorption capacity of PE, PP and PS for different POPs (i.e. PAHs, hexachlorocyclohexanes and chlorinated benzene) showed that PS had the highest sorption capacity following by PE and PP, respectively [Lee et al.; 2014]. Other than transporting and releasing the contaminants, plastic can also modulate the POPS and heavy metals toxicity both enhancing or reducing their effects. MPs increased the accumulation of Hg in brain and muscle of *Dicentrarchus labrax* marine fish causing neurotoxicity (through the inhibition of acetylcholinesterase [AChE]) and increased lipid peroxidation. Moreover, MPs / Hg mixture changed differently the activity of Lactate Dehydrogenase [LDH] and Isocitrate Dehydrogenase [IDH] based on the concentrations of compounds [Barboza L. G. A. et al.; 2018]. In mice, co-exposure of PE or PS MPs and Organophosphorus Flame Retardants [OPFRs] caused neurotoxicity and oxidative stress, enhancing the Superoxide Dismutase [SOD] and Catalase expression in liver, compared to the single OPFRs treatment [Deng Y. et al.; 2018]. On the other hand, adsorption of PAHs by PS NPs decreased the POPs uptake by Zebrafish larvae and reduced the fish development defects [Trevisani R. et al.; 2019].

Heavy metals and POPs result from natural environment composition and/or human activities, such as high levels of PAHs are observed in oil fields [Hirai H. et al.; 2011]. Both the concentration of pollutants and the time of exposure influence their adsorption on plastic surface. Increasing level of heavy metals (e.g. Pb and Zn) on PET, PVC, LD-PE and PP were observed in a time-dependent manner during a 12-months study period [Rochman C. M. et al.; 2014].

2.4. Toxicity of Plastics related to Microorganisms

Microorganisms may colonize the plastic debris due to their high surface area. Plastic items act as vectors that can carry microbes toward other places, exposing them to several organisms. This biofilm named “platisphere” is potentially dangerous since it may include pathogenic, toxic invasive and plastic-degrading organisms (such as toxic microalgae) and antibiotic-resistant or metal-resistant bacteria [Prata J. C.; 2018]. The ingestion or inhalation of microbes that grow on MPs may change the microbiomes of the organisms altering the physiological functions and threatening the host health [Wright S. L. et al.; 2017]. Moreover, the “platisphere” alters the plastic density changing the plastics distribution, their buoyant capability and promoting their sinking to the marine sediments [Caruso G.; 2019].

Diverse biofilms were evaluated in different areas of the North Sea during different seasons on PET bottles,

suggesting the principal role of the local physical-chemical characteristics for the development of microbe community [Oberbeckmann S. et al.; 2016]. Furthermore, a recent study showed the influence of the plastic properties (e.g. hydrophobicity, roughness and electrostatic interaction) for the initial bacteria colonization. Specific plastic type-bacteria strains were analyzed in the biofilms developed on LD-PE, HD-PE, PP and PVC after one week in the Northern Adriatic (e.g. PVC was colonized by bacteria of the *Alteromonadaceae*, *Cellvibrionaceae* and *Oceanospirillaceae* probably due to the presence of phthalates) but, the differences among the bacterial community decreased after two months of incubation [Pinto M. et al.; 2019].

3. Laws and Regulations

3.1. Policies on Plastics

Since the plastic pollution is dangerous for the environment as well as for the organisms, including the human health, worldwide countries are enacting laws and regulations to reduce the plastic consumption leading to a lower production. Moreover, they promote recycling and recovery policies following the 3Rs principle (Reuse, Recycle and Recover) to obtain economic benefits. Limitations on plastics are primarily applied on single-use plastic objects (i.e. plastic bags, straws and cotton swabs) due to their greater diffusion as environmental pollutants on the beaches and in the seas [EUR-Lex; 2019].

Plastic bags are banned in several countries all over the world. Since 2014, eight American states have totally banned single-use plastic bags (i.e. California, Hawaii, New York, Connecticut, Delaware, Maine, Oregon and Vermont), while cities in other states have imposed fees on the bags to reduce their use (e.g. a $0.05 fee is applied on all plastic bags in the city of Washington) [Our American States – The NCSL (National Conference of States Legislatures) Podcast; 2020 - Californian Against Waste (CAW) Recycles; 2019]. National actions regulate the plastic bags use within the European countries, since the absence of an International directive. For example, Denmark, Germany, Greece, Hungary, Ireland, Norway, Netherlands, Portugal, Spain, Sweden and United Kingdom have taxed the carrier bags minimizing their consumption [Lam C. S. et al.; 2018 – European Commission; 2013]. On the other hand, the introduction of fees on plastic bags did not reduce their consumption in South Africa, probably because the population accepted to pay the tax [Lam C. S. et al; 2018]. However, a new regulation on carrier bags have been proposed by the Department of Environmental Affairs of South Africa [Environmental Affairs, Republic of South Africa; 2017].

Single-use plastic objects such as dishes, forks, knives, spoons, straws, cotton swabs, balloons and balloons sticks will be banned since 2021 in Europe by the introduction of the EU directive 2019/904 [EUR-Lex; 2019]. Single-use straws and PS containers have been restricted in Vermont since 2019 and the ban on PS has been applied in the city of Hastings-On-Hudson (New York state) since 2014 [Our American States – The NCSL (National Conference of States Legislatures) Podcast; 2020].

Recently, some countries have focused on plastic microbeads, that are commonly used in personal care products and whose release in the environment is difficult to avoid, due to their little dimensions. The “Microbead-Free Water Act 2015” has banned the microbeads in cosmetics in the United States [U.S.] and the “Ocean Plastic Charter” will reduce the use of microbeads in cosmetic and personal care products (e.g. soaps, toothpaste, scrubs) by 2020 in Canada, France, Germany, Italy, the United Kingdom and the European Union [H.R.-1321; 2016 – Ocean Plastic Charter; 2018].

Recycling and recovery policies are promoted by the countries to both reduce the plastic pollution and improve the economy through the development of new industrial sectors (e.g. waste treatment and research of plastic alternatives) toward a “circular economy” [EUR-Lex; 2019]. For example, the International directives “The Ocean Plastic Charter” and the “European Strategy for Plastics in Circular economy” pursue to reduce the plastics in the seas and oceans increasing the recycling and recovery processes [Ocean Plastic Charter; 2018 – EU; 2018]. In Italy, the recent “*Decreto Salva Mare*” allows the fishermen to bring the plastics waste collected in the sea to the land for their recycling [Ministero dell’Ambiente; 2019]. However, the recycling and recovery of plastics are still matter of debate since the

composition of the materials and the presence of additives that may inhibit their reprocessing [Hopewell J. et al.; 2009]. Alternatively, energy recovery allows to produce energy through the plastic incineration, although pollutants may be released, such as dioxins and metals [North E. J., Halden R. U.; 2014]. Moreover, countries promote the research of materials (e.g. bioplastics) that could be totally degrade in the environment, as replacement of the non-degradable plastics [EUR-Lex; 2019].

Finally, national and international organizations enact several campaigns and events to sensitize the public opinion. For example, the "Beat Plastic Pollution" movement founded by World Meteorological Organization [WMO], the World Health Organization [WHO] and the United Nations Environment Programme [UNEP], has been involved in several campaigns to decrease the plastic pollution such as "CleanSeas" (www.cleanseas.org) and "StopSucking" (www.plasticfreeme.org/stopsucking) [WMO; 2018].

3.2. Legislation on Additives

Numerous additives are added during the plastic production process to improve the yield of the reaction and the material properties. Since they are not bound to the polymer matrix, additives could migrate from the plastic to the medium in contact with the object and so toward the environment, influenced by the storage conditions, such as temperature and time of exposure [Hahladakis J. N. et al.; 2018]. Evidences of additives migration into food and food simulants are described in table 4.

Table 4: Overview of studies that analyzed the additives migration from plastics to foods based on Hahladakis J. N. et al.

Packaging material/type	Migrated substance	Food / Food Simulant [FS]	Storage: Temp (°C)	Storage: Contact time	Comments - Findings
PS cups	Styrene	Distilled water	60, 40, 20, 4	3 d	Styrene migration was influenced by fat content and storage temperature of food, exhibiting higher migration levels in in hot beverages than in the cold ones.
		Distilled water	100	1, 2 h	
		Milk	100, 60, 40, 20	2 h	
		Milk	40, 20, 4	24 h	
		Milk	4	3 d	
		Juice	20	16 h	
		Jelly, pudding	4	1, 3, 7 d	
		Hot beverage	100	1 h	
		Drinking chocolate	20	16 h	
		Cola, beer	20	16 h	
		Ice-cream	-10	30, 60 d	
		Drinking yogurt	4	3, 7, 14 d	
PS	DEHA Styrene, overall migration	Iso-octane	40	2 h	For overall migration iso-octane is an alternative FS. For DEHA each of the FSs should be considered
		Yogurt, dessert	25	8-28 d	

					separately. Styrene migration was in all cases higher than ethylbenzene. In addition, longer contact time and higher fat content favoured migration.
PS, PP, PE, PET	Relative migration	Vegetable pure oil 3% (v/v) aqueous acetic acid, 15% (v/v) ethanol, and olive oil	5	10	PS caused the fastest migration in olive oil while PET had the highest migration in the FS 15% ethanol.
PVC	DHA, HOA, HAD, DEHA and overall migration	Sliced ham	25	0, 1, 3, 5, 10, 30, 45 min	HDA demonstrated higher migration in ham after 45 min, while the migration was found to be proportional to fat content of material and contact time.
		Olive oil	40	10 d	
PVC	Ethylbenzene, DEHA	Yogurt, dessert	25	8-28 d	Kefalotyri exhibited the highest level of migration followed by Edam and Feta.
		Kefalotyri, Edam and Feta cheese	5	1-240 h	
PVC	DEHA	Cheese	40	2 h, 1 d	DEHA migration was higher at 21°C after 5 d. Lowest migration was observed at -5°C after 2 h.
			21	2h, 1, 5 d	
			5	2 h, 1, 5, 10 d	
PVC	DHA, HOA, HDA	Cheese	25	5 min	Migration increased with increasing fat content.
LD-PE	Irganox 1076	Ethanol	28-60	-	No influence of the FS type on the transport properties into the plastic films were observed; thus, no absorption of the FS into the plastic tested films occurred in this work.
LD-PE	Irganox 1076	Cheese sauce, chicken, chocolate, margarine, milk, mayonnaise, milk, orange juice, pork,	40	30 d	The highest level of migration was observed in chocolate (32.1% fat): 1413 µg dm^{-2}.

		salmon, wheat flour, FSs (distilled water, 3% acetic acid, ethanol 10%, rectified olive oil, iso-octane, 95% ethanol)			
rPET	Toxic metals	5% aqueous citric acid or deionized water	1700 W or 7.2-22.2	5 min or 1, 7, 14 d	Neither the storage nor the MW treatments had significant effect on metal migration. Exposure to 5% citric acid resulted in a higher rate of leached metals compared to deionized water.
Melamine Formaldehyde	Overall migration	3% (w/v) acetic acid	25, 800 W	1, 2, 3 or 5 min (repeated heating cycles)	MW heating for 1-2 min over long-term use created concern. Service terms in a MW oven were drastically reduced, by more than 10-fold compared to conventional heating.
Retall packaging material	HA, DBP, BHT, Cyanox 2246, Chimassorb 81, Irganox 1035 / 1010 / 1330 / 1076, Irgafos 168, Tinuvin 326 / 328	FS-A, B, C, D	40	10 d	Low-molecular weight compounds were detected in aqueous simulants. Irganox 1010 and 1330 were found in oil simulants (respective range: 20.28-330.44 µg g^{-1}, 3.08-47.31 µg g^{-1}). Detected concentrations in aqueous FS = BHA: < Limit of Quantification; DBP: <14.43 µg g^{-1}; BHT: <706.3 µg g^{-1}; Cyanox 2246: <20.68 µg g^{-1}; Irganox 1035: <2.03 µg g^{-1}.
PVC gasket	ESBO, DEHP, DINP, DIDP, DEHA, DEHS, ATBC	Oily food (Olive, mussels in oil, tuna in oil, etc.)	120, 150, 40	1, 4, 10 d	Average Migration was 46% for DEHA, ATBC and DEHA, while ESBO showed the higher transfer with 90% of migration.
				10 d	
PET	Tinuvin 234	Miglyol (coconut oil	40-70	10 d	Migration of Tinuvin 234 from PET was very slow:

		simulant), water/ethanol solution, iso-octane			2 µg dm^{-2} in 95% ethanol.
Plastic container	Phthalates	Cooking oil and mineral water	20, 40, 60	60 d	Cooking oil proved to be a more suitable medium for phthalate migration than mineral water. Higher temperatures and longer contact time favoured migration.
Tablewares as drinking utensils	Phthalates	Drinking water	100	1 h	Concentrations of DBP and DEHP in the drinking water samples (10.13 ng mL^{-1} and 5.83 ng mL^{-1}) exceeded the limit levels for drinking water (8 ng mL^{-1} and 3 ng mL^{-1}).
Plastic baby bottles	Overall migration	50% ethanol	70	2 h	PP bottles showed the greater number of migrated substances. Migration rate = DiBP and DBP: 50–150 µg kg^{-1}; DEHP: 25 - 50 µg kg^{-1}; DINP: 25 µg kg^{-1}; 2,4 DTBP: 400 µg kg^{-1}.
Plastic vs non-plastic packaging material	BPA	Olive oil	25	1 y	Higher BPA levels were measured in oil samples stored in plastic vs non-plastic packaging materials. Estimated exposure was 1.38% of the EFSA tolerable daily intake, thus no concerns arose of potential health risks from olive oil consumption.
Epoxyphenolic coated can	BPA	Soup, minced beef, evaporated milk, carrots, 10% ethanol	5, 20, 40	1, 3, 9 months (5-20°C); 10 d, 1, 3 months (40°C)	80-100% of BPA migrated to food during the can filling. BPA migration amount = 10% ethanol: 68.3 ± 9.0 µg kg^{-1}; minced beef: 53.8 ± 7.6 µg kg−1; milk: 49.8 ± 10.9 µg kg^{-1}; carrots: 47.2 ± 5.1 µg kg^{-1}; soup: 45.7 ± 5.0 µg kg^{-1}.

PC and other plastic containers (PC baby bottles, non-PC baby bottles, baby bottle liners, and reusable PC drinking bottles)	BPA	Water, 10% ethanol, 50% ethanol	40	240 h	Higher temperatures and longer treatment periods resulted in higher BPA migration from PC bottles. The average concentration of residual BPA in 50% ethanol was higher (2.39 µg L^{-1}) compared to water (1.88 µg L^{-1}).
LD-PE	DPBD	Chicken, pork	5, 25	10 d	High storage time and temperature favoured migration. No significant differences were observed between the two temperatures tested.
PVC	DEHA, ATBC	Sesame paste	25	0.5-240 h	ATBC at equilibrium was found to be approx. 2.5 times lower than DEHA which can be attributed to lower initial concentration of ATBC (1.8 mg dm^{-2}) in the film, compared to that of DEHA (3.2 mg dm^{-2}).
Cup, plate, container meat tray	Styrene	Oil	70	10 d	Cup exhibited the highest migration levels of all other material at 150°C (1.39 µg cm^{-2}) after 10 d of exposure.
LD-PE, PVC LD-PE, PS LD-PE, PP	Oleamide, Erucamide, Stereamide	FS-A, B, C, D	40	10 d	Polyolefin exhibited the highest amount of migration (88% for oleamide, 98% for erucamide, 95% for stearamide). Slip compounds were almost totally migrated from 65 µm LD-PE film, whereas PVC or PS exhibited miniscule migration (<1% of total).
LD-PE	BHA, DBD, BHT, Irganox 1010 / 1076, Irgafos 168, Ethanox 330	Distilled water	60 40 ± 1	20 d 10 d	Of all migrated substances studied, only Irgafos 168 and Ethanox 330 were detected in FS.
PA, PE/PA, PP	Overall migration	Olive oil, ethanol 95%	40	10 d	For PA/PE, 95% ethanol appeared to be the best alternative fatty FS. For

					PP, isopropanol and n-heptane yielded almost the same amount of migration.

[ATBC] = Acetyltributyl citrate; [BHA] = 2- and 3-t-butyl-4-hydroxyanisole; [BHT] = Butylated Hydroxytoluene; [BPA]= Bisphenol A; [DBP] = Dibutyl Phthalate; [DEHA] = Di-(2-ethylhexyl) Adipate; [DEHP] = Bis (2-ethylhexyl)phthalate; [DEHS] = Diethylhexyl succinate; [DEP] = Diethyl Phthalates; [DHA] = Diheptyl Adipate; [DiBP] = Diisobutylphthalate; [DIDP] = Diisodecyl Phthalate; [DINP] = Diisononyl phthalate; [DPBP] = diphenoxybenzophenone; [2,4 DTBP] = 2,4-di-tert-butyl phenol; [EFSA] = European Food Safety Authority; [ESBO] = Epoxidized Soybean Oil; [HAD] = Heptyl Adipate; [HOA] = HOA Heptyl Octyl Adipate; [MW] = Microwave; "Food Simulant A" = 10% ethanol (v/v); "Food Simulant B" = 3% acetic acid /w/v); "Food Simulant C" = 20% ethanol (v/v); "Food Simulant D" = 50% ethanol (v/v); "-" = not information available [adapted from Hahladakis J. N. et al.; 2018].

As previously described, several additives are identified as medium-high hazardous compounds, hence their use is limited by national and international laws; while the low dangerous additives are mainly not classified and regulated [Rodrigues M. O. et al.; 2019]. For example, BPA as well as phthalates, that are recognized as "Endocrine Disrupting Chemicals" [EDCs], have been limited in several countries, while the BPA analogue (BPS) is not regulated. BPA has been banned in the production of bottles for infants and children in Europe, Canada, China and U.S. [EUR-Lex; 2018 – Canada Consumer Product Safety Act; 2010 – Congressional-Executive Commission of China (CCEC); 2011 - The NCSL (National Conference of States Legislatures); 2015]. In Europe, plastic materials, such as PVC and polyurethanes, can contain the following phthalates DEHP, DBP, BBP and DIBP at a maximum concentration of 0.1% by weight [EUR-LEX; 2018]; although, the phthalates have been banned in the production of toys for children in Europe, Canada and U.S. [Hazardous Product Act (HPA); 2010 - Consumer Product Safety Improvement Act (CPSIA); 2008 - EUR-Lex; 2005].

Regulations such as, the European "Registration, Evaluation, Authorisation and Restriction of Chemicals" [REACH], the Canadian "Chemical Management Plan" [CMP] and the American "Toxic Substances Control Act [TSCA] Modernization Act of 2015", limit the use of additives and define their application in different materials highlighting their risks, in order to reduce their potential toxic effects on human and environment [EUR-LEX; 2009 – CMP; 2016 – TSCA; 2015]. Furthermore, additives migration limits for food packaging and the experimental conditions to test them have been established in the European "EU No 10/2011" [EUR-Lex; 2011]. For example, migration limits of the plasticizers BBP, DBP and DEHP have been set for the production of plastic materials in contact to only non-fatty food (BBP = 30 mg kg^{-1}, DBP = 0.3 mg kg^{-1} and DEHP = 1.5 mg kg^{-1}), for which their use is permitted. While migration is forbidden or should not be detectable (less than 0.01 mg kg^{-1}) for other substances, e.g. 1,3-butadiene [EUR-Lex; 2011].

However, a specific additives regulation may be difficult to establish since different factors influence their concentration within the plastics and their potential release in the environment, such as the material compositions and the abiotic / biotic fragmentation processes.

3.3. Legislation on Water Contaminants

Regulation on environmental pollutants, concerning especially water contaminants, is of interest since the

capability of plastics to adsorb heavy metals and POPs on its surface increasing their potential exposition to organisms, including human [Rodrigues J. P. et al.; 2019].

National and international policies have been acted to reduce the release of contaminants in the sea and oceans. The "Convention for the Protection of the marine environment of the North-East Atlantic" (OSPAR Convention) has been signed by the states of the North -East Atlantic Oceans (e.g. Belgium, Ireland, Iceland and UK) aiming to reduce the POPs, PAHs and Hg emissions [OSPAR; 2010]. Moreover, 152 worldwide countries approved the "Stockholm Convention on Persistent Organic Pollutants" in 2004. This international environmental accordance currently counts 184 parties (183 states and European Union) and regulates the use and emission of 28 POPs, including organo-chloride pesticides (e.g. aldrin and DDT) and industrial chemicals (e.g. PCBs and dioxins) [United Nations; 2020 – Earth Negotiations Bulletin (ENB); 2019]. Several legislations have been introduced since 2004 to apply the directives of the "Stockholm Convention", such as the European REACH Regulation which controls the chemicals use and application, the Regulation No 689/2008, the Council Directives 96/59/E and 2000/76/EC that limit respectively the export of POPs, the use of PCBs and the emissions of dioxins/furans in Europe [EU; 2009]. Among the chemicals, Hg is strongly regulated since its toxicity, actually Hg and Hg-containing products are totally banned in Europe by the REACH, as well as Hg use and export bans have been introduced in U.S through the "Mercury Export Ban Act" [EUR-Lex; 2009 – Public Law 110-4141; 2008].

Moreover, the WHO has suggested guide values for the maximum concentration of POPs and heavy metals in the waters considering also the contribution of chemicals natural sources, that are described within the "Guidelines for drinking water quality, 4th edition" (Table 5) [WHO; 2011].

Table 5: POPs and heavy metals concentrations in water based on the "Guidelines for drinking water quality, 4th edition".

Compound	**Maximum Concentration (mg/L)**	**Comments**
Arsenic [As]	0.01	Up to 12 mg/L in areas with natural sources of As.
Boron[B]	2.4	0.5 mg/L for drinking water.
Cadmium [Cd]	0.003	
Chromium [Cr]	0.05	2 µg/L in drinking water.
Copper [Cu]	2	
Lead [Pb]	0.01	5 µg/L in drinking water.
Mercury [Hg]	0.006	
Nickel [Ni]	0.07	Usually less than 0.02 mg/L in drinking water.
Uranium [U]	0.03	
Aldrin and Dieldrin (pesticides)	0.00003	Considering their combination.
DDT	0.001	
Benzo(a)pyrene	0.0007	Usually 0-5 ng/L in uncontaminated water.

[DDT] = Dichlorobiphenyl Trichloroethane [WHO; 2011].

4. Guidelines for proper Mussels and Shellfish Consumption

The Food and Agriculture Organization of the U.S. [FAO] estimated a global seafood consumption over the 151 million tons in 2016, including 18 million tons of aquaculture molluscs (e.g. shellfish), and resulting in an apparent consumption of 20.3 kg of fish and fishery products per person per year [FAO; 2018]. Marine organisms could ingest plastic items and particles identifying them as food and suffering of the plastic and related contaminants (additives, POPs and heavy metals) toxicity. Plastic particles were evaluated both in pelagic and benthic fish species of the Adriatic Sea: these researches showed high variability among the location and the type of fish, suggesting a different MPs ingestion due to the feeding habitats and the conditions of the location (Table 6) [DeFishGear; 2016]. Although, contrasting studies described differences in MPs ingestion by benthic and pelagic fish, MPs should be considered as dynamic particles that can sink to the sediment (available to the benthic species feeding) and potentially be remobilized to the water column returning to be accessible also for the pelagic species [DeFishGear; 2016]. Moreover, the urbanization of the location may influence the plastic pollution and hence, the potential ingestion by marine organism in accordance to their carnivore/herbivore habits; for example, evidences showed a more frequently ingestion of plastic by the carnivore species [Azevedo-Santos V. M. et al.; 2019].

Table 6: MPs evaluation in fish and mussel of the Adriatic Sea based on "DeFishGear project" – IPA Adriatic Cross-border Cooperation Programme 2007-2013.

Fish / Shellfish	Habitat	Country	Average MPs / fish, mussel	Comments
Mullus surmuletus	Benthic	Croatia	2.68	Filaments were more than fragments.
Pagellus erythrinus	Benthic		1.88	
Sardina pilchardus	Pelagic		2.54	
Mullus barbatus	Benthic	Greece	0.88	Fragments were more than filaments.
Pagellus erythrinus	Benthic		1.20	
Sardina pilchardus	Pelagic		2.53	
Mullus barbatus	Benthic	Bosnia and Herzegovina	12.95	Filaments were more than fragments.
Pegusa lascaris	Benthic		9.45	
Gobius cobitis	Benthic		8.4	
Sprattus sprattus	Pelagic		5.35	
Mytilus galloprovincialis (mussel)	Benthic	Bosnia and Herzegovina	3.67	Only filaments were observed.
	Benthic	Greece	3.15	Same ratio of filaments and fragments.
	Benthic	Italy	1.65	Fragments were more than filaments.

Fish species: *Mullus barbatus, Mullus surmuletus, Gobius cobitis, Pegusa lascaris, Sprattus sprattus*. Mussel: *Mytilus galloprovincialis* [DeFishGear; 2016].

Trophic transfer to plastics from prey to predators may be hypothesized [Zhang S. et al.; 2019]. Hence,

plastics particles may reach the human following the food chain, through the fish and molluscs consumption, and exerting their potential toxic effects on human health.
This deliverable aims to describe the currently data on plastic and related contaminants ingestion by seafood, focusing on mussels and shellfishes in order to suggest guidelines for their proper consumption.

4.1. Currently Mussels and Shellfish Consumption

Shellfish production is increasing since they are becoming a very popular seafood item. Mussels, scallops, clams and oysters are relatively cheap, easy to cook and rich of nutrients, such as protein, vitamins (A and D) and essential minerals (Iodine, Selenium, Calcium) [GLOBEFISH; 2019]. For example, mussels are rich in long chain polyunsaturated fatty acids (e.g. eicosapentaenoic acid [20:5n-3 EPA] and docosahexaenoic acid [22:6n-3 DHA]) with anti-inflammatory properties. The oil of *Perna canaliculus* mussel reduced the incidence and progression of metabolic-osteoarthritis in oil mussel-treated rats compared to a high fat/high sugar diet [Siriarchavatana P. et al.; 2019].
Most of the shellfish is farmed: actually only the 6% of the total is captured, while the remaining 94% is supplied by aquaculture [Danovaro R. et al.; 2004]. Globally, clams and oysters contribute respectively for the 38% and 33% to the entire production, while scallops and mussels take part respectively for the 17% and 13% to the global request [Wijsman J. W. M.; 2018]
Production of mussels increased in the period from 2007 to 2016, reaching the 2 million tons in 2016. China was the principle producer (879,000 tons) and Europe contributed with 522,000 tons; China and Europe together account for the 67% of the global mussel production in 2016. In Europe, Spain was the largest producer (200,000 tons per year), followed by Italy, France, the Netherlands, Denmark and Germany. The more cultured mussel species differ among the country producers:
i) Mediterranean *Mytilus galloprovincialis* mussel (producers: Italy, Spain, France and Greece);
ii) Green Mussel (*Perna viridis*; producers: Thailand and Philippines);
iii) New Zealand Green Mussel (Perna canaliculus; producer: New Zealand);
iv) Chilean Blueshell Mussel (*Mytilus chilensis*; producer: Chile);
v) Korean Mussel (*Mytilus coruscus*; producer: Korea).
Focusing on the partners involved in the NET4mPLASTIC project, shellfish production in Croatia includes the Mediterranean *Mytilus galloprovincialis* mussel and the European flat *Ostrea edulis* oyster and it is based especially on farming: actually, 746 tons of mussels and 52 tons of oysters were cultured in 2015 in farms located in the western-coast of Istria, estuary of Krka and Novigrad Sea [FAO Croatia; 2020]. In Italy, shellfish farms are located mainly in the Adriatic region (e.g. Emilia-Romagna, Marche and Veneto regions) where the Mediterranean *Mytilus galloprovicnialis* mussel and the Japanese *Ruditapes philippinarum* carpet shell are the major cultured species. In 2013, 64,235 tons of mussels and 24,609 tons of carpet shell, including Japanese carpet shell, were produced that accounted respectively to the 72% and 28% of Italian shellfish production other than the 95% of the carpet shell production in Europe. Oyster production is marginal with only 53 tons in 2013 [FAO Italy; 2020].
Regarding the consumption, the apparent European mussels request was of 577,000 tons in 2016 and the 75% of which was consumed by Spain, France and Italy showing peaks of consumption related to the seasons and the locations (e.g. larger consumption was observed during summer in tourist areas). Moreover, Italy was the largest mussel market in 2016 with an apparent mussel consumption of 120,000

tons provided from both own production and imported mussels [European Market Observatory for Fisheries and Aquaculture products [EUMOFA]; 2019]. Since, Spain, France and Italy account the major mussel consumption representing only the 35% of European population, the other European countries should consume the remaining 25% of mussels, which hypothetically results in 200 g of mussel per capita per year [Monfort M. C.; 2014].

4.2. Occurrence of Environmental Chemical Contaminants in Mussels

Mismanaged waste from land enhances the contaminants contribution within the oceans together to their voluntary released by human and their involuntary transport by the weather (e.g. wind) and the river flows. As much as 80% of marine plastic litter is originated from land and about the 5% of the total is carried by the river [Jambeck J. R. et al.; 2015 - Kataoka T., Nihei Y.; 2020].

Mussels are benthic filter feeders that can pump and filter large amounts of water (rate of 50 mL of seawater per minute) through the coordinated action of cilia localized at the gill epithelium surface, exposing themselves to the chemical and organic pollutants of the oceans and seas [Barker Jørgensen C.; 1990 – Famme P. et al.; 1986]. Since the worldwide distribution, the easiness of the sampling and the accumulation of contaminants, mussels are commonly used in the environmental monitoring of metals and POPs [Li J. et al.; 2019].

Several studied analyzed PCBs, PAHs and heavy metals in mussels from the Adriatic Sea (Table 7). For example, metals (i.e. Hg, Ni, Cu, Zn, Cd, Cr, Pb and Vanadium [V]), organochlorides pesticides, PAHs and PCBs were evaluated in *Mytilus galloprovincialis* collected in 38 stations along the Adriatic coast, resulting in high concentrations of pollutants especially in urban and industrialized areas, e.g. Taranto appeared as the most contaminated site for both Hg and PCBs [Bajt O. et al.; 2019].

Table 7: Chemicals detected in marine organisms collected from the Adriatic Sea.

Species	Chemicals detected	Sampling point	n. sampling sites	Sampling period	References
Mussels	PCBs, PAHs, metals (Hg, Ni, Cu, Zn, Cd, Cr, Pb, V)	Eastern and Western Adriatic Sea	38	2008 (from May to July)	Baji O. et al., 2019
Mussels	PCBs, metals (Cd, Cr, Cu, Hg, Pb, Zn)	Eastern Adriatic coast	14	2006 (March)	Kljakovi-Gašpic Z. et al.; 2010
Bivalves, molluscs	PCBs, Trace metals	Croatian Adriatic coast	11	2012 (May and November)	Milun V. et al.; 2016
Mussels	PCBs, PAHs, metals (Fe, Mn, Cu, Zn, Co, Ni, Cd, Pb, Hg)	Boka Kotorska Bay	7	2009 (winter, spring)	Jovic M, Stankovic´ S.; 2014
Bivalves, cephalopods, crustaceans,	PAHs	Central Adriatic Sea (Abruzzo)	-	2004 (July and December)	Perugini M. et al.; 2007

fish					
Mussels	PBDEs, PCBs	Apulia region coast	32	2008 (September and October)	Giandomenico S. et al.; 2013
Molluscs, crustaceans, fish	PCDDs, PCDFs, PCBs	Western Adriatic Sea	19	1997-1998 (from April to June - from November to January)	Bayarri S. et al.; 2001

Cd] = Cadmium; [Co] = Cobalt; [Cu] = Copper; [Fe] = Iron; [Hg] = Mercury; [Mn] = Manganese; [Ni] = Nickel; [Pb] = Lead; [Zn] = Zinc; [PAHs] = Polycyclic Aromatic Hydrocarbons; [PCBs] = Polychlorinated Biphenyls; [PCDDs] = Polychlorinated Dibenzodioxins; [PCDFs] = Polychlorinated Dibenzofurans; [PBDEs] = Polybrominated Diphenyl Ethers.

Currently, the European regulation (EC) No 1881/2006 establishes the maximum limits of contaminants (i.e. heavy metals, dioxins, PCBs and PAHs) in fishes and fishery products, including bivalve molluscs (Table 8) [EUR-Lex; 2006].

Table 8: Maximum concentrations of heavy metals and POPs for molluscs based on (EC) No 1881/2006 regulation.

Pollutant	**Seafood**	**Maximum Limit**
As	Molluscs	1.00 mg/kg
Cd	Molluscs (excluding queen scallops and oysters)	2.00 mg/kg
	Bivalve molluscs	1.00 mg/kg
Hg	Molluscs	1.50 mg/kg
	Bivalve molluscs	0.50 mg/kg
Pb	Molluscs	2.00 mg/kg
	Bivalve molluscs	1.50 mg/kg
dioxins + dioxin like-PCBs	Bivalve molluscs	4.00 pg/g wet weight
PCBs	Bivalve molluscs	8.00 pg/g wet weight
benzo(a)pyrene	Bivalve molluscs	10.00 µg/kg

[As] = Arsenic; [Cd] = Cadmium; [Hg] = Mercury; [Pb] = Lead; [PCBs] = Polychlorinated Biphenyls [EUR-Lex; 2006].

Microplastics have been recently recognized as contaminants of emerging concern in seafood and, their evaluation in mussels has been suggested as a parameter for the marine health status [Vandermeersch G. et al.; 2015 - De Witte B. et al.; 2014]. PS nanoparticles decreased the filtering activity of *Mytilus Edulis* mussels and altered the expression of biotransformation, DNA damage and immune response genes (e.g. increased expression of cytochrome P450-1-like-1 [cyp11] and decreased levels of cytochrome P450-3-like-2 [cyp32], p53 and lysozyme) in *Mytilus galloprovincialis* [Wegner A. et al.; 2012 – Brandts I. et al.; 2018]

Laboratory studies evaluated the uptake, accumulation and toxicity of MPs in mussels showing plastic

debris ingestion in all the tested concentrations and the accumulation of the smaller fragments (up to 80 µm) in the digestive system within hours (Table 9). While, larger MPs were commonly egested as faeces or pseudofaeces [Li J. et al.; 2019].

Table 9: Uptake and accumulation of MPs by mussels in laboratory tests based on Li J. et al.

Exposure microplastic			**Exposure Concentration**	**Exposure Time**	**Uptake and Accumulation organs**
Type	**Shapes**	**Size**			
Mytilus edulis					
HD-PE	powders	0-80 µm	2.5 g/L	96 h	gill, stomach, digestive gland
PS	spheres	3-9.6 µm	42 particles/L	3 h - 48 d clearance	gut, haemolymph
	particles, beads	100 nm, 10 µm	$1.3x10^4$ particles/mL and 1000 beads/mL	45 min - 72 h clearance	digestive gland
	beads	30 nm	0.1, 0.2, 0.3 g/L	8 h	foot
	spheres	10, 30, 90 µm	110 particles/mL	14 d - 24 h clearance	whole soft tissue
	beads, fragments, fibres		100, 1000 particles/L	5 d	whole soft tissue
	fibres		2000 microfibres/L	48 h - 48 h clearance	gill, intestine, foot, stomach, mantle, gonad, adductor, visceral tissue
PS, PE, PP	beads, fibres	7-30 µm (beads) or 23 x 3000 µm (fibres)	50 beads/mL or 0.1 fibres/mL	60 min	whole soft tissue
Mytilus galloprovincialis					
PS, PE	powders	<100 µm	1.5 g/L	7 d	haemolymph, gill, digestive gland
LD-PE	particles	20-25 µm	$2.34x10^7$ particles/L	28 d	haemolymph, gills, digestive glands, intestine
PE	fragments (derived from toothpaste)	50-590 µm	0.01 g/L	21 d	digestive tract, whole boy
PS	spheres	3 µm	$50\text{-}1x10^4$ particles/mL	24 h - 192 h clearance	gut of larva
Mytilus spp.					
PS	beads	2, 6 µm	32 µg/L/day - 2000	7 d - 7d clearance	digestive tract intestine, gills

			beads/mL/day		
Dreissena polymorpha					
PS	beads	1, 10 µm	$1x10^6$ or $4x10^6$ particles/L	6 d	gut, digestive gland, haemolymph
Geukensia Demissa					
PS, PE	spheres	5, 250-300 µm	3.467 g/L	2 h - 24 h clearance	stomach, digestive tubules, intestine
Perna perna					
PVC	spheres	0.1-1 µm	0.5 g/L	3 h - 12 d clearance	gut, haemolymph

[HD-PE] = High-Density Polyethylene; [LD-PE] = Low-Density Polyethylene; [PE] = Polyethylene; [PP] = Polypropylene; [PS] = Polystyrene; [PVC] = Polyvinyl chloride [Li J. et al.; 2019].

Moreover, depuration mechanism has been demonstrated to reduce the MPs content in plastic-treated mussels. Both wild and farmed mussels showed similarly reduction in MPs content (about the 33% of the total) when kept in an aquarium without feeding for stomach depuration [Birnstiel S. et al.; 2019].
According to mussel feeding strategies and laboratory exposure studies, pathways on MPs uptake, accumulation and egestion have been hypothesized. When MPs in seawater encounter the mussel's gill surface, they may be captured and trapped into mucus and subsequently assimilated over the gill epithelium or transported into the mouth and the digestive system [Von Moos N. et al.; 2012; Kolandhasamy P. et al.; 2018]. However, not every captured particle is ingested, since mussels are able to separate and reject non-nutritive particles as pseudofaeces, in order to defend the organism from the high quantities of suspended particulate matter [Santana M. F. et al.; 2018]. Smaller particles are ingested and retained in mussels more easily compared to the larger particles suggesting that mussels select for a specific size range of MPs during ingestion and egestion process. For example, only the smallest particles (10 µm) were detected in mussels exposed to three different sizes of MPs (10, 30, 90 µm) [Van Cauwenberghe L. et al.; 2015].
Other than size, the age and the concentration of plastics influence their uptake by mussels. Environmentally aged MPs are differentially ingested, e.g. the major uptake of PE-weathered MPs (PE particles from toothpaste) was evaluated in MPs-exposed *Mytilus galloprovincialis* mussels, in comparison to virgin MPs [Bråte I. L. N. et al.; 2018]. Moreover, accumulation of MPs in mussels increased in a dose-dependent manner in organisms treated with different concentrations of beads/fragments/fibres [Qu X. et al.; 2018]. It should be highlighted that in many laboratory studies, organisms are exposed to unrealistically high doses of MPs with uniform size or shape, in virgin condition and for relatively short time frames. Whereas, environmental plastics are subject to weathering, abrasion and photodegradation, leading to a broad size distribution and various shapes. In order to perform more realistic experiments mimicking environmental weathering, some studies exposed organisms to MPs collected from beaches or deployed in a bay for a period time [Bråte I. L. N. et al.; 2018]. Finally, the comparison among MPs concentration in mussels collected along the Chinese coast and MPs uptake by mussels in laboratory (mussels were exposed to beads, fibres or fragments for 5 days) showed a major amount of fibres in capture organisms, while beads were the most ingested particles during experimental treatments [Qu X. et al.; 2018]. Probably, beads may be more easily ingested by mussels in short time periods; while fibres

accumulation may result from long-term exposure in the marine environment, since their more difficulty to be removed from mussels' gills and hepatopancreas after their deposition [Qu X. et al.; 2018 – Renzi M. et al.; 2018].

Depuration significantly decreased the quantity of MPs in mussels identifying the digestive system as the probable deposit of the eliminated particles. However, many particles were still detected after depuration performed up to 7 days (i.e. 3 – 7 days), suggesting that MPs could have been translocated to other tissues or even to the circulatory system, or that the tested depuration times may not have been long enough to completely eliminate them [Van Cauwenberghe L., Janssen C. R.; 2014 – Paul-Pont I. et al.; 2016 - Browne et al.; 2008].

4.3. Human MPs Ingestion through Mussels Consumption

Since their filtering activity, mussels are vulnerable to plastic pollution and can act as vectors for MPs transfer into the food chain, potentially exposing human to plastic, additives and environmental contaminants [Conti I. et al.; 2021].

Consumption of mussels is characterized by the ingestion of the whole organism contained within the shells, including the digestive tract, which has been hypothesized as the main location for MPs deposit [Van Cauwenberghe L., Janssen C. R.; 2014 – Renzi M. et al.; 2018]. Notwithstanding the average number of plastic fragments in bivalves is 0.2-4.0 items/g [EFSA; 2016], several studies evaluated different MPs contents probably related to the diverse analyzed marine organisms and the sampling locations (Table 10). For example, from 0.26 to 0.51 fibres/g of individual were detected in mussels (i.e. Mytilus edulis, Mytilus galloprovincialis and Mytilus edulis/galloprovincialis hybrid form) collected from Belgian coasts and purchased from Belgian supermarkets [De Witte B. et al.; 2014]. A maximum value of 1.1 particles/g of was evaluated in mussels sampled at six locations along the French-Belgian-Dutch coastline (average 0.2 ± 0.3 particles/g of mussel) [Van Cauwenberghe et al.; 2015], while the highest number of MPs (i.e. 4 particles/g) was observed within the Chinese shellfish [Li J. et al.; 2015]. Moreover, the extraction of MPs from food can be performed using different methods that could influence the results, such as plastics extraction using HNO_3 caused the total degradation of nylon fibres [EFSA; 2016].

Table 10: Occurrence of MPs in mussels.

Bivalves	Sampling Location	MPs average content	References
Mytilus edulis	3 Belgian supermarkets, Belgian groynes (3 locations) and quaysides (3 locations)	0.37 particles/g wet weight	De Witte B. et al.; 2014
Mytilus edulis	Mussel farm in North Sea	0.36 particles/g wet weight	Van Cauwenberghe L. and Janssen C. R.; 2014
Commercial bivalves (9 species)	Fish market in China	4.0 particles/g wet weight (range 2.1-10.5 particles/g)	Li J. Et al.; 2015
Mytilus edulis	6 locations along French-Belgian-Dutch coastline	0.2 ± 0.3 particles/g wet weight	Van Cauwenberghe L. et al.; 2015

Mytilus galloprovincialis	Mussel farms in Italy	6.2-7.2 particles/g wet weight	Renzi M. et al.; 2018
Mytilus galloprovincialis	Mussel farms in Bosnia and Herzegovina	3.67 particles/individual	DeFishGear; 2016
Mytilus galloprovincialis	Mussel farms in Greece	3.15 particles/individual	
Mytilus galloprovincialis	Mussel farms Italy	1.65 particles/individual	

Assuming 250 g as average portion of mussels (without shell), a person may ingest from 50 to 1,000 particles using the MPs average numbers established by EFSA (0.2 and 4 items/g, respectively). However, a recent study observed a reduction in the putative MPs intake of about the 14% per meal, in case the mussels were cooked and the cooking water was not consumed. The high temperature during cooking was hypothesized to determine micro-fractures or fusion of the plastic polymers, inducing fragmentation of plastic items and their release in the cooking water [Renzi M. et al.; 2018]. In this view, the previous calculated amount of ingested MPs (from 50 to 1,000 particles per portion of mussels) would be reduced to 43 and 860 MPs per portion of mussels, respectively. Considering the MPs as spherical particles with an average particle size diameter of 25 µm and a density of 0.92 g/cm^3 (density of LD-PE, the most common polymer type of MPs) [EFSA; 2016], these 43-50 and 860-1,000 plastic particles would represent about 0.3-0.35 µg and 6.0-7.5 µg of plastics, respectively.

Furthermore, important implications for human health derived also both from the additives that are added to the plastic during the manufacturing process, and from the environmental contaminants that can be adsorbed by the plastic items [Renzi M. et al.; 2018]. Generally, additives contribute for about the 4% of the weight of the plastics [Bouwmeester H. et al.; 2015], meaning that 0.3-0.35 / 6.0-7.5 µg of MPs would contain respectively 0.012-0.014 / 0.24-0.30 µg of additives (4% of the total weight of plastics). Finally, up to 2,750 ng/g of PCBs and up to 24,000 ng/g of PAHs were evaluated in MPs deposited at beaches [EFSA; 2016]. Assuming that these POPs would be completely released from the plastic items, the previous calculated µg of ingested MPs (i.e. 0.3-0.35 / 6.0-7.5 µg) would lead to the assumption of 0.82-0.96 / 16.5-20.6 pg of PCBs and 7.2-8.4 / 144-180 pg of PAHs (Table 11).

Table 11: MPs, additives and POPs ingestion through mussel consumption.

Average MPs content / g of mussel wet weight	Mussels Processing	MPs per portion	Additives per portion (µg)	PCBs per portion (pg)	PAHs per portion (pg)
0.2	Raw	50 particles (0.35 µg)	0.014	0.96	8.40
	Cook	43 particles (0.30 µg)	0.012	0.82	7.20
4.0	Raw	1,000 particles (7.50 µg)	0.300	20.60	180.00
	Cook	860 particles (6.00 µg)	0.240	16.50	144.00

Assuming 250 g as the average portion of mussels per person (without shells).
[PAHs] = Polycyclic Aromatic Hydrocarbons; [PCB] = Polychlorinated Biphenyls.

The shellfish consumption changes among the countries leading to different MPs, additives and related contaminants putative ingestion. Average consumptions of 1.87 Kg of mussels/inhabitant/year and 200 g of mussel/inhabitant/year have been estimated for Italy and Croatia, respectively [Monfort M. C.; 2014]. Assuming both the minimum and the maximum MPs content (0.2 and 4.0 MPs/g of raw mussel wet weight), MPs, additives and POPs ingestion per capita per year would be respectively: 40-800 plastic particles, 0.012-0.24 µg of additives, 0.82-16.5 pg of PCBs and 7.2–144 pg of PAHs per inhabitant per year in Croatia; 374-7,480 plastic particles, 0.112-2.240 µg of additives, 7.70-154 pg of PCBs and 67.20–1344 pg of PAHs per inhabitant per year in Italy (Table 12).

Table 12: MPs, additives and POPs ingestion through mussel consumption (raw or cooked mussels) in Croatia and Italy.

Country	Average MPs / g of mussel wet weight	Mussels Processing	MPs	Additives (µg)	PCBs (pg)	PAHs (pg)
Croatia (200 g / inhabitant / year)	0.2	Raw	40 particles (0.3 µg)	0.012	0.82	7.20
		Cook	34.4 particles (0.26 µg)	0.010	0.71	6.24
	4.0	Raw	800 particles (6 µg)	0.240	16.50	144.00
		Cook	688 particles (5.1 µg)	0,204	14.02	122.40
Italy (1.87 kg / inhabitant / year)	0.2	Raw	374 particles (2.8 µg)	0.112	7.70	67.20
		Cook	321.6 particles (2.4 µg)	0.096	6.60	57.60
	4.0	Raw	7,480 particles (56 µg)	2.240	154	1,344.00
		Cook	6,432.8 particles (48.2 µg)	1.929	132.55	1,156.80

Estimation of MPs, additives and POPs ingestion per inhabitant per year based on consumption of mussels listed by Monfort M. C. [Monfort M. C.; 2014].
[PAHs] = Polycyclic Aromatic Hydrocarbons; [PCB] = Polychlorinated Biphenyls.

5. Conclusions

Plastic pollution is a worldwide problem concerning both the environment health and the organism wellness, including human. Despite their social benefits, the increased use of plastics coupled to their non-degradation property and mismanaged policies have resulted to enhance plastic waste accumulation in lands and oceans.

Marine organisms could ingest plastic objects and microparticles confusing them as food and suffering of their toxic effects. Other than of the chemical and physical properties of the polymer itself, toxicity of plastic is related to both the additives added during the polymerization reaction (e.g. phthalates, bisphenols, benzene) and to the environmental contaminants that can be adsorbed on plastic surface (i.e. POPs and heavy metals). Moreover, ingested microparticles and related compounds can enter in the food chain reaching and potentially exerting their toxic effect on human.

As filtering organisms, mussels pump several amounts of seawater exposing themselves to the plastic contamination as consequence of the MPs ingestion and accumulation in mussels' tissues. Since mussels are becoming a very popular seafood due to their availability and nutritional properties, human consumption of mussels would cause the assumption of plastics, additives and related contaminants.

Several countries are introducing bans and directives in order to limit the plastics use aiming to reduce the plastic pollution. Moreover, the actual regulations on additives and water contaminants may be implemented, since the high hazardousness of these compounds and their close relations with plastic particles. Only a comprehensive view on plastics, additives and water contaminants might suggest political actions to decrease the environmental pollution and the human exposure to these potentially toxic substances. However, further studies will be required to evaluate the trophic transfer of MPs and related additives / POPs / heavy metals from seafood (i.e. mussels) to human and to analyze their toxicity on human health.

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