# Chapter 6 Weight of Evidence for the Microplastic Vector Effect in the Context of Chemical Risk Assessment



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Abstract The concern that in nature, ingestion of microplastic (MP) increases exposure of organisms to plastic-associated chemicals (the 'MP vector effect') plays an important role in the current picture of the risks of microplastic for the environment and human health. An increasing number of studies on this topic have been conducted using a wide variety of approaches and techniques. At present, the MP vector effect is usually framed as 'complex', 'under debate' or 'controversial'. Studies that critically discuss the approaches and techniques used to study the MP vector effect, and that provide suggestions for the harmonization needed to advance this debate, are scarce. Furthermore, only a few studies have strived at interpreting study outcomes in the light of environmentally relevant conditions. This constitutes a major research gap, because these are the conditions that are most relevant when informing risk assessment and management decisions. Based on a review of 61 publications, we propose evaluation criteria and guidance for MP vector studies and discuss current study designs using these criteria. The criteria are designed such that studies, which fulfil them, will be relevant to inform risk assessment. By critically reviewing the existing literature in the light of these criteria, a weight of evidence assessment is provided. We demonstrate that several studies did not meet the standards for their conclusions on the MP vector effect to stand, whereas others provided overwhelming evidence that the vector effect is unlikely to affect chemical risks under present natural conditions.

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#### 6.1 Introduction

Plastics in the environment contain mixtures of chemicals. These chemicals stem from deliberate additions during manufacture of the plastic or from the ambient water via absorption or both. Plastic particles are known to be ingested by organisms, including humans (World Health Organization 2019), but not all particles can be ingested by all organisms. Exposure, bioavailability, feeding behaviour and size of the plastic item in relation to size of the mouth opening determine whether a plastic item can be taken up (Jâms et al. 2020; Koelmans et al. 2020). Plastic is persistent, and ingestion of sufficiently high particle concentrations has been demonstrated to cause effects on small organisms like some zooplankton and invertebrates, most likely due to the reduced caloric value of the ingested material (Gerdes et al. 2019a; de Ruijter et al. 2020). Besides this mechanism of reduced caloric value due to dilution of ingested material, concerns have been raised due to the fact that chemicals on the plastic are ingested with the plastic. This has led to an increasing number of studies that investigate the potential of microplastic to increase exposure of organisms to plastic-associated chemicals. Henceforth, we will refer to these studies as MP vector studies. MP vector studies have used a wide variety of approaches and techniques and have been summarized in several reviews (Rochman 2015, 2019; Koelmans et al. 2016; Ziccardi et al. 2016; Hartmann et al. 2017; Burns and Boxall 2018). However, none of these have focused on quality assurance and study design criteria.

In the recent literature, MP vector studies are often framed as 'controversial', 'complex' or 'under debate'(Gassel and Rochman 2019). This suggests that apparently there is no consensus in the scientific community on the nature or the relevance of the MP vector effect. We argue that there are three main reasons why this could be the case, reasons that may explain why it takes so long before consensus is reached.

The first reason relates to confusion about when a study is to be considered relevant in this context, a question which in the literature on chemical risks often is referred to as the 'so what' question. To date, studies have addressed detailed mechanisms, specific exposure scenarios in the lab, have either measured effects on uptake or on biological endpoints in the lab and have evaluated uptake under natural conditions either by field studies or by model scenario analysis. Studies mostly addressed whether chemicals can (potentially) be taken up from plastic under some specific conditions, but not if they will be taken up under natural conditions. Few studies have strived at interpreting results in the context of environmentally relevant conditions, and none of them addressed to what extent ingestion of MP would actually increase the chemical risks for organisms. An increase in chemical risk would exist if, due to the MP vector effect, exposure to chemicals (e.g., predicted exposure concentrations (PEC)) would exceed the toxicity thresholds that are known for these chemicals (predicted no effect concentration (PNEC)). Putting study results in the context of such actual risks is relevant, because if a detected vector effect would not increase risks of the chemicals, then it might be less important to address it and there would be less reason for concern. With no studies actually demonstrating the occurrence of an MP vector effect on chemical risks under natural conditions, the evidence base is remarkably thin. This constitutes a research gap, because these are the conditions that would be most relevant when informing risk assessment and management decisions. We thus propose relevance for chemical risk assessment as an overarching umbrella criterion to evaluate the setup of MP vector studies.

The second reason is that the discussion of the topic may have lacked a common understanding of the processes at play, especially in the earlier studies. Most of them address a part of the exposure conditions that are relevant to the occurrence of MP vector effects and their possible implications for effects on risks in the environment (e.g. Rochman et al. 2013; Chua et al. 2014; Wardrop et al. 2016; Granby et al. 2018; Kühn et al. 2020). Only few studies use theoretical frameworks to add greater depth to the interpretation of study outcomes. Such frameworks allow for inter- and extrapolation across chemical properties, microplastic characteristics, biological traits and environmental conditions (Koelmans et al. 2016; Bakir et al. 2016; Rochman et al. 2017; Lee et al. 2019; Mohamed Nor and Koelmans 2019; Wang et al. 2020a, b). As these frameworks often come in the form of mathematical models, they are not automatically adopted by researchers that use laboratory or field observational studies as their primary research tools.

The third reason relates to the general quality of some of the microplastic research, which has been framed as limiting in recent literature (Lenz et al. 2016; Hermsen et al. 2018; Koelmans et al. 2019; Markic et al. 2019; de Ruijter et al. 2020; Provencher et al. 2020). Microplastic research is a young and fast-growing field in the environmental sciences, and this is reflected in the wide variety of approaches used when sampling and analysing microplastic and in effect testing methods. The same diversity and therefore incomparability of approaches apply to MP vector studies.

In this review, we discuss the potential of studies and study designs to inform risk assessment of plastic-associated chemicals, emphasizing organic chemicals. Heavy metals bind to microplastic particles as well, but their sorption affinity is limited compared to that for sorption to natural particles like sediment (e.g. Besson et al. 2020). First, we propose evaluation criteria for MP vector studies. The criteria are designed such that studies that fulfil them can be considered relevant to inform risk assessment. Second, we critically review the existing literature in the light of these criteria, thereby providing a weight of evidence assessment. Finally, we discuss several key references from the literature as examples of how they can be used to inform plastic-associated chemical risk assessment given the criteria and recommendations.

Literature was selected through reference as well as cited reference searches for the aforementioned six existing MP vector effect reviews. This method assumes that all relevant MP vector studies either cite or are cited (in) at least one of these six reviews, published between 2015 and 2019.

We emphasize that we reviewed the existing literature only for the aim set for the present review: to assess the weight of evidence for the chemical vector effect to occur under environmentally relevant conditions and with respect to implications

for chemical risks. Part of the reviewed papers may not have had this specific aim. They often were performed to verify the validity of underlying mechanisms, or they were meant to study *potential* effects rather than to demonstrate actual chemical risks under field conditions. Therefore, the present retrospective assessment does not necessarily disqualify studies as such, as they could have had other aims than mimicking the environmental realism with respect to the chemical risks stemming from the MP vector effect that we aim to pursue here.

# 6.2 Guidance for Microplastic Vector Studies in the Context of Chemical Risk Assessment

Several criteria can be used when interpreting results from studies investigating the MP vector effect with respect to their relevance for conditions occurring in nature (Table 6.1). The criteria can be placed into two broad categories: (a) characteristics of a study in providing evidence for the occurrence of an MP vector effect in nature and (b) relevance of the setup and outcomes for risk assessment of chemically contaminated MP. The difference is that the latter category would need reflection on whether effect thresholds for chemical toxicity are exceeded. For instance, if an MP vector effect is detected, concentrations of environmentally relevant chemical mixtures still may be too low to exceed such toxicity threshold concentrations. Furthermore, even if ingestion of plastic would increase actual risks because the plastic acts as a source for some plastic-associated chemicals, the same ingested plastic could at the same time act as a sink ('cleaning agent') for chemicals other than these plastic-associated ones, i.e. chemicals which are present in the gastrointestinal tract from other sources (Koelmans 2015). An increasing number of studies has demonstrated that this is possible, from empirical evidence as well as through model scenario analysis (Koelmans et al. 2013b, 2016; Devriese et al. 2017; Scopetani et al. 2018; Mohamed Nor and Koelmans 2019; Heinrich and Braunbeck 2020; Thaysen et al. 2020). In terms of risks of the overall chemical mixture that animals are exposed to, the cleaning phenomenon could compensate for the increased exposure of plastic-associated chemicals, possibly leading to net zero or even less effects of the chemical mixture. Below, we further detail these criteria for laboratory studies, field studies, in vitro studies and modelling studies.

#### 6.2.1 Criteria for In Vivo Laboratory Studies

An often used setup of laboratory MP vector studies is that MP is first contaminated with chemicals, after which test organisms are exposed to these contaminated MPs and to controls without MP for comparison. An MP vector effect is then indicated if body burdens are higher in the MP treatments than in the control, whereas a risk is

| Cri | terion   | Guidance  | Type of study<br>for which the<br>criterion is<br>relevant <sup>a</sup> |
|-----|--|---|---|
| 1   | Preparation of<br>microplastic with<br>associated<br>chemicals | Use long-term field-contaminated microplastic, or<br>laboratory spiking with equilibration time of at least a<br>month, and/or corrections for non-equilibrium by<br>means of kinetic sorption modelling  | L   |
| 2   | Resemblance of<br>natural exposure<br>pathways                 | Chemical exposure via all pathways that are relevant<br>to the organism under consideration is assessed, either<br>through measurement or modelling, preferably<br>through both   | L, F, M   |
| 3   | Verification of<br>chemical exposure                           | Chemical exposure should be assessed for all exposure pathways, either through measurement or modelling   | L, F  |
| 4   | Concentration gradient   | No or limited concentration gradient; if the study aims<br>to mimic natural conditions<br>Maximum gradient; if the study aims to assess<br>sorption kinetic parameters  | L, M  |
| 5   | Ingestion  | Ingestion should be demonstrated  | L, F  |
| 6   | Evidence from correlations                                     | Rule out multiple causation<br>Assess correlations on the level of individual<br>chemicals<br>Account for measurement error   | F   |
| 7   | Reversibility of chemical transfer                             | Parameter fitting and data interpretation should<br>account for bidirectional chemical transfer between<br>ingested plastic and biota tissue  | L, F, M   |
| 8   | Model validity   | The model needs to be consistent with empirical data, with current knowledge, and with design criteria  | М   |
| 9   | Threshold effect concentration                                 | If a microplastic vector effect is detected, it should be<br>assessed whether it leads to exceedance of a chemical<br>threshold effect concentration  | L, F, M   |
| 10  | Mixture toxicity   | If a microplastic vector effect is detected, it should be<br>assessed whether the increased ('vector effect') and<br>decreased ('cleaning effect') chemical exposures due<br>to microplastic ingestion still lead to a net exceedance<br>of a chemical threshold effect concentration for the<br>chemical mixture | L, F, M   |

 Table 6.1
 Quality criteria for studies investigating the microplastic vector effect in the context of chemical risk assessment

 $^{a}L$  laboratory study, F field study, M model study

indicated if the increased exposure is the reason for exceeding an effect threshold. Several factors define the weight of evidence from such studies (Table 6.1).

**Preparation of MP with Associated Chemicals** Several studies have spiked chemicals, e.g. persistent organic pollutants (POPs) to MP, in order to mimic environmentally relevant MP. This should be done for long enough time, because in the environment the far majority of microplastic particles has an age in time scale of

more than months. For instance, Rochman et al. (2013) deployed MP pellets in San Diego Bay for 3 months, which yields environmentally relevant concentrations that are in (near-)equilibrium with the ambient water. Equilibrium has been argued to be the most relevant state for POPs on MP (Koelmans et al. 2016; Lohmann 2017; De Frond et al. 2019; Seidensticker et al. 2019) and implies that the chemicals are relatively well diffused inside the polymer phase, rendering desorption to be slower compared to non-equilibrium situations. After all, when POPs are pre-sorbed for short times only, they desorb from outer sorption domains, which is faster. This means that any detected vector effect would be overestimated. Sorption times as short as 72 h have been applied in some studies (e.g. Kleinteich et al. (2018), Chua et al. (2014)) which thus would lead to higher desorption rates than in nature. In principle, such non-equilibrium artefacts can be corrected via modelling, where the parameters obtained from a non-equilibrium setup can be used in a scenario analysis for equilibrium conditions (Mohamed Nor and Koelmans 2019; Seidensticker et al. 2019).

Sometimes it is argued that additives can have higher than equilibrium concentrations, suggesting that the aforementioned long sorption times would not be needed for such studies. However, microplastic is, by definition, a mixture of the smaller plastic in the environment, largely originating from slow fragmentation, with time scales longer than chemical desorption time scales. The default state for additives thus also is chemical equilibrium with ambient water (Koelmans et al. 2016; Lohmann 2017; De Frond et al. 2019; Seidensticker et al. 2019), a condition which is not always met in laboratory studies mimicking scenarios with additives. For a credible study, we advise an equilibration time of a month or longer, or corrections by means of kinetic sorption modelling when shorter times are used.

Resemblance of Natural Exposure Pathways In the environment, exposure of organisms to chemicals occurs via multiple pathways, simultaneously (Fig. 6.1). For aquatic organisms, the most important pathways are dermal uptake via skin and/ or gills, and water and food ingestion, whereas for air-breathing organisms, inhalation can be relevant. If MP is present, ingestion of MP might contribute to total uptake. If this occurs, it does not necessarily imply that it is a significant vector. It has been demonstrated that transfer of chemicals via ingestion of MP often is negligible compared to the sum of the uptake via the other pathways (Koelmans et al. 2013b; Bakir et al. 2016). Recent experimental studies have confirmed this experimentally, e.g. for lugworms (Besseling et al. 2017), seabirds (Herzke et al. 2016), daphnids (Horton et al. 2018), marine phytoplankton and zooplankton (Beiras et al. 2018, 2019; Beiras and Tato 2019; Sørensen et al. 2020). These observations comply with theory as was shown in several studies where experimental and natural conditions were simulated using numerical modelling (Bakir et al. 2016; Lee et al. 2019). In contrast, some of the key studies in the literature did not address other exposure pathways (e.g. Browne et al. (2013); Rochman et al. (2013); Chua et al. (2014); Wardrop et al. (2016)). These studies have only one possible outcome, namely, that the ingestion of microplastic is important for chemical uptake because other exposure mechanisms that would occur in nature have been disabled. These



**Fig. 6.1** Diagram illustrating the uptake and elimination pathways for chemicals by and from fish. PCB is used to represent chemicals in general. Processes A, B, C and D represent the concomitant terms in Eq. (6.1)

studies have provided support for the MP vector effect paradigm, even though they did not necessarily seek relevance with respect to natural conditions. While the data from these studies are relevant under the conditions used, their deviation from natural conditions makes them less useful for risk assessment related to the MP vector chemical effect. In summary, experimental designs should ideally include, quantify and discuss all pathways relevant in nature, either based on measurement or on measurement in combination with modelling.

Control, Measurement and Assessment of Exposure Multiple pathways thus should be included in MP vector study designs, and these should be quantified in order to assess the relative importance of the MP vector effect. One approach is to assess this via measurement. This requires assessment of chemical concentrations in plastic, water and ingested food throughout the experiment (e.g., Besseling et al. 2017; Rehse et al. 2018; Gerdes et al. 2019b). Many studies have interpreted data based on nominal concentrations of chemicals on plastic, which however is not fully reliable. After all, when chemicals are spiked on the plastic as described in the previous section, or when they also are present in added food, they can (partly) desorb or re-equilibrate before MP ingestion has taken place. This implies that the original, nominal concentrations on plastic do not apply anymore, and actual exposure is unknown. This hampers data interpretation and has led to uncertainty with respect to the applicability of the results of some studies. This especially occurs for studies that used gradient between MP sorbed with chemicals and clean water (Rochman et al. 2013; Chua et al. 2014; Wardrop et al. 2016; Beckingham and Ghosh 2017). In a recent review, Burns and Boxall (2018) re-analysed exposure in a study where Oryzias latipes were exposed to MP associated with organic contaminants sorbed from San Diego Bay (Rochman et al. 2013) and assessed that there was insufficient chemical mass on the MP to explain mass measured in fish for most chemicals, suggesting that the cod oil in the diet also was a source. MP vector studies also have suggested that chemicals evaporated from their experimental systems, further limiting data interpretation (Rochman et al. 2017). There are established approaches to measure aqueous phase concentrations, such as with passive samplers, and these have already been applied in some MP vector studies (Besseling et al. 2017; Horton et al. 2018; Wang et al. 2019).

The second approach to assess the relative magnitude of the MP vector effect compared to competing uptake pathways is by calculation. Several studies have demonstrated how results from experiments that did not as such fully comply to natural conditions can still be used in model scenario analysis that were environmentally relevant (Koelmans et al. 2016; Rochman et al. 2017). An example of such an assessment is provided as a case study in Section 4 of this review.

Resemblance of Chemical Concentration Gradients as They Would Occur in Nature Several studies have mentioned non-equilibrium exposure as a limitation in the interpretation of their data (e.g. Beckingham and Ghosh 2017; Sleight et al. 2017; Rochman et al. 2017). Furthermore, it has been argued that chemical equilibrium is the most likely state for chemicals on small microplastic (Koelmans et al. 2016; Diepens and Koelmans 2018; De Frond et al. 2019; Seidensticker et al. 2019). Microplastic is small by definition and 'old' for the far majority of the particles, because its main process of formation is the slow process of embrittlement, erosion and fragmentation. Smaller and older particles are closer to equilibrium, due to shorter intrapolymer diffusion path lengths and longer sorption time scales (Seidensticker et al. 2019). For microplastic, sorption kinetic time scales range from weeks to months. Residence times of the particles in the environment are long (90% being older than 2 years in the ocean (reviewed in Koelmans et al. (2016)), which is one of the main reasons for the concerns surrounding microplastic. In combination, these factors cause equilibrium to be the default state for plastic-associated chemicals. The same applies to other small particles in the ocean, like detritus, black carbon and sediment or suspended solid organic matter, which all would have sorption half-lives of days to weeks at most (Schwarzenbach et al. 2005). This however also applies to organisms at the base of the food chain, as well as, for instance, fish eggs and larvae. In later stages of their life cycle, fish consume contaminated prey keeping the organism body burden close to equilibrium or even higher due to biomagnification. This implies that for chemicals in ingested microplastics, there is no concentration gradient that would drive transfer to the organism, to begin with. For MP vector studies to be environmentally relevant, this means that plastic, organisms, water and other compartments (food, sediment) should all include a scenario where chemicals are contaminated to the same extent (equal fugacity), ideally by pre-equilibration of the chemicals prior to exposure to microplastic. Although second best, studies could still use a large enough gradient, in order to be able detect an effect in the first place. However, in such a case, results should be calculated back to a natural conditions scenario. Alternatively, the bias caused by testing chemical uptake from contaminated MP by clean organisms can be balanced by also testing the opposite treatment, i.e. by using (relatively) clean MP ingested by contaminated test organisms (Koelmans et al. 2013b; Rummel et al. 2016; Scopetani et al. 2018; Wang et al. 2020b). Studies that make an effort to use pre-equilibrated exposure media and organisms (e.g. Besseling et al. (2013); Besseling et al. (2017)) or that otherwise account for vector as well as cleaning mechanisms combined, score higher, whereas evidence from studies that, for instance, force chemical transfer by using clean test animals would receive lower weight.

*Assessment of Ingestion* Laboratory MP vector studies have been performed with a wide variety of test organisms, covering functional groups such as phytoplankton, zooplankton, (benthic) invertebrates and fish. The essence of the MP vector hypothesis relates to chemical transfer upon ingestion of the contaminated particles. Therefore, we argue that MP vector studies should ideally provide evidence that the test animals actually ingested the particles and preferably also at which rate.

#### 6.2.2 Criteria for Field Studies

Studies have attempted to use observed co-occurrences or correlations between field data on plastic densities or of chemical concentrations in plastic, with chemical concentrations in organisms, as evidence supporting the MP vector hypothesis. The main challenge here is that, whereas in laboratory experiments typically all conditions are kept the same except for the one being researched, in nature, (a) multiple mechanisms are at play simultaneously, (b) there is no control over them, and (c) they may remain partly unknown. Given this, several factors define the weight of evidence from such studies (Table 6.1).

Resemblance of Natural Exposure Pathways Various researchers and expert groups have defined multiple processes and uptake pathways that can explain the occurrence of chemicals (e.g. POPs, PBTs, additives) in organisms, one of which is by ingestion of MP and subsequent chemical desorption inside the organism (GESAMP 2015; Rochman 2015; Koelmans et al. 2016). This means that studies should motivate why the uptake flux via these combined other parallel processes is small compared to the chemical uptake flux via microplastic ingestion. Pathways should be assessed such that it is relevant for the organism studied. However, studies have often neglected to consider such parallel pathways. For instance, the effect of microplastic ingestion on bioaccumulation of phthalates by the fin whale was speculated from detection of plastic in plankton samples and from phthalates being detected in the same plankton samples and fin whale (Fossi et al. 2012). However, the plankton to microplastic number concentration ratios were higher than 1000. Given these high ratios and residence times of microplastic in the oceans, causing chemical desorption from microplastic and subsequent uptake by plankton, it is most likely that dermal absorption and ingestion of phthalate-contaminated plankton were the dominant pathways, rather than from direct ingestion of the microplastic. A follow-up study analysed the case using theoretical modelling and indeed found that 99.2 % of phthalate uptake was by plankton ingestion and only 0.8 % by microplastic ingestion (Panti et al. 2016). Another example is the study by Tanaka et al. (2013), who found some chemicals in seabirds that were absent in prey and thus concluded that the ingested plastic must have been the source. However, the prey organisms in which the chemicals were not detected were collected 7 years after the year of collection of the seabirds, rendering the comparison inconclusive (Burns and Boxall 2018; Tanaka et al. 2013). There are few field studies that guantify the fluxes of the parallel pathways based on field data (Koelmans et al. 2014; Herzke et al. 2016; Panti et al. 2017), which all conclude a negligible relevance of the MP vector effect. As an opposite example, for a 'hot spot' location in the North Pacific accumulation zone, Chen et al. (2017) calculated MP mass to biomass ratios and determined that MPs outweigh prey in the same size range (0.5-5 mm) by 40 times (and by 180 times if all buoyant plastic and biota > 0.5 mm are considered). They thereby suggested that MP at the surface may make up a significant dietary contribution. A further example of such an analysis is provided as a case study in Section 4. In summary, like empirical studies, field studies should account for all exposure pathways in order to assess the relative importance of the MP ingestion vector effect, either based on measurement or on measurement in combination with modelling.

*Evidence from Correlations* Some studies have found evidence for the MP vector effect in observed correlations between field data on plastic densities or of chemical concentrations in plastic, with chemical concentrations in organisms (Rochman et al. 2014; Panti et al. 2017; Gassel and Rochman 2019). There are a few pitfalls with the use of such correlations. First, correlations show that variables are related, but they do not reveal the causality of the relationship. For example, in the oceans, high plastic concentrations with high chemical concentrations will inevitably lead to high chemical concentrations in the seawater and thus in biota, via phase partitioning. These concentrations thus will always be correlated. However, this also occurs without ingestion, thus minimizing the weight of evidence from such correlations. Given that (a) MP ingestion is not often verified (Koelmans et al. 2016), (b) studies that assessed ingested MP reveal that MP levels are very low (Foekema et al. 2013; Hermsen et al. 2017; Markic et al. 2019), and (c) if they would be ingested, a gradient for transfer would be lacking (see above), chemical accumulation via the water or ingestion of zooplankton is a far more plausible explanation. Furthermore, bioaccumulation is driven by many factors and not by plastic density alone. For instance, phytoplankton blooms or dissolved organic carbon (DOC) concentrations are highly dynamic and have been demonstrated to affect chemical concentrations in the oceans (Dachs et al. 2002; Jurado et al. 2004). Bioaccumulation thus is multifactorial and without considering all factors at play, i.e. by using univariate correlations, no conclusions on implications of MP can be drawn. Co-occurrence or correlations of high chemical concentrations in fish with higher plastic density are difficult to interpret if plastic densities apply to the surface, whereas the fish species reside (for considerable time) in another habitat, for instance, below the surface, like for myctophids (e.g. Gassel and Rochman 2019). Another pitfall relates to the use of summed concentrations of the investigated chemicals (e.g. **SPCBs**; Rochman et al. 2014; Gassel and Rochman 2019). The sums all have a different proportion of each of the individual chemicals in the mixture. Therefore, each data point in the correlation resembles something different, another variable, rendering the correlation to be unclear. Instead, we argue that for an unambiguous measurement of chemical trends, such correlations should only use concentrations of the same one chemical. A final evaluation criterion relates to accounting for error and uncertainty in the data used in the correlation. As mentioned, the dependent variable, which is often the summed chemical concentration, suffers from uncertainty related to the variability in congener composition which is not accounted for. However, regression analysis, as used in these types of studies, considers the independent variable (e.g. MP density) as if it contained no error itself. This is problematic when these data points actually are prone to error, which is often the case. For instance, Gassel and Rochman (2019) correlated sum chemical concentrations with plastic concentrations interpolated by modelling, which can be assumed to have considerable uncertainty. In these cases, it is recommended that weighted regression procedures are applied. In studies that use such correlations, such pitfalls are typically not discussed. In summary, we recommend that studies that provide evidence based on correlations, to rule out parallel causal explanations for the observations, use analyses of individual chemicals and take measurement error into account.

*Assessment of Ingestion* Similar to laboratory MP vector studies, field studies that conclude that ingestion of MP increase chemical concentration in biota should provide evidence that the test animals actually ingested the particles and preferably also at which rate.

# 6.2.3 Criteria for In Vitro Studies

Several scientists have studied chemical transfer kinetics between plastic particles and water or plastic particles and gut fluids or stomach oil (e.g. Teuten et al. (2007); Bakir et al. (2014); Beckingham and Ghosh (2017); Lee et al. (2019); Martin and Turner (2019); Mohamed Nor and Koelmans et al. (2019); Kühn et al. (2020)). Besides the common quality assurance (QA) criteria that apply to any study that needs the analysis of chemical concentrations on plastic, there are several criteria that need to be fulfilled in order to make a study relevant with respect to risks caused by the MP vector effect (Table 6.1). Chemical desorption kinetics in artificial gut fluids have been assessed in order to show the MP vector effect. Such studies should either use long-term adsorption prior to desorption in order to not overestimate the desorption rates, as mentioned in Section 2.1, or the level of non-equilibrium should be accounted for in the desorption kinetic parameter estimation (Mohamed Nor and Koelmans 2019). Because sorption involves a dynamic equilibrium, desorption

studies should also account for backward sorption in their setup and data analysis. Kinetic modelling to acquire the rate constants may need to take intrapolymer diffusive mass transfer and/or biphasic behaviour into account (Seidensticker et al. 2017, 2019; Town et al. 2018; Lee et al. 2019). Kinetic parameters should be provided with significance level and/or error estimate, preferably using multiple time points in the desorption curve allowing for a rigorous estimation of parameter values (Mohamed Nor and Koelmans 2019). Furthermore, besides exchange fluxes between MP and gut fluid, the fluxes from food should be assessed in order to assure that the role of ingested MP is not negligible as compared to the parallel exposure pathways. For instance, Kühn et al. (2020) observed leaching of five chemicals from MP into stomach oil sampled from seabirds. However, the uptake of chemicals from normal food and the concentrations of background chemicals in the oil were not measured or modelled, nor were kinetic parameters estimated, precluding conclusions regarding the relevance of the vector effect under environmentally realistic conditions. Furthermore, the in vitro transfer data cannot be assumed relevant for natural conditions in vivo where removal processes such as metabolization and elimination would reduce the gradient for transfer and where bioaccumulation by seabirds is likely to be at steady state. The kinetic data thus have to be put in the context of all relevant processes that govern the uptake and elimination of the chemicals of interest. Models have been developed that allow for such a context-dependent evaluation using the kinetic parameters obtained from in vitro desorption studies (Bakir et al. 2016; Koelmans et al. 2013b, 2016). Studies that account for all these aspects would be most relevant for assessing the relevance of the MP vector effect in the context of chemical risk assessment.

#### 6.2.4 Criteria for Model Scenario Studies

Several studies have applied models to calibrate parameters relevant for the MP vector effect (Koelmans et al. 2016; Mohamed Nor and Koelmans 2019) or to evaluate the importance of the MP vector effect based on empirical data (Gouin et al. 2011; Koelmans et al. 2013b, 2014, 2016; Bakir et al. 2016; Herzke et al. 2016; Rochman et al. 2017; Wang et al. 2019; Wang et al. 2020a, b). As a starting point for the evaluation of studies that used models, we adopt the criteria for a valid model suggested by Rykiel Jr (1996): 'Validation is establishing the truth of a model in the sense of (a) consistency with data, (b) accordance with current knowledge, (c) conformance with design criteria'. Consistency with data means that MP vector models should be demonstrated to concur with observations. This implies that calibrated parameters comply with theory or that parameter sets purely obtained from theory comply with observations. Model frameworks that lack such consistency would score lower. Accordance with current knowledge means that the model application or parts of it should not be in conflict with established and widely accepted evidence-based theoretical concepts or that no such concept is overlooked in case it would be relevant. For instance, model scenario studies informing chemical risk assessments about the relevance of the MP vector effect should account for reversible exchange in the gut, should take into account what is the gradient occurring under natural conditions and should account for all pathways that are relevant under natural conditions. Conformance with design criteria means that a model's complexity, approach and output comply with what a model is meant to do in a certain context. For instance, a model should not be over-parameterized or should it be too simple with respect to the dominant processes that drive the behaviour of the system under consideration.

# 6.3 Weight of Evidence Supporting the Microplastic Vector Hypothesis in the Context of Chemical Risk

The literature provides varying types of evidence for the MP vector effect and its implications on chemical risks. In 2016, we provided a first evaluation of 13 key laboratory, field and model studies that were available at that time (Koelmans et al. 2016). The evaluation was based on whether studies used realistic MP concentrations or MP fractions in the diet, whether MP ingestion was confirmed and whether all environmentally relevant uptake processes were accounted for. In 2018, a similar analysis was published by Burns and Boxall (2018), who evaluated 18 studies and ranked them into 3 evidence categories: 'demonstrated', 'inconclusive' and 'not supported'.

The review in this section can be seen as an extended and detailed version of these earlier evaluations. Here, we evaluate the weight of evidence for the MP vector effect from 61 studies, based on 2 criteria: (a) is the evidence conclusive enough, given the recommendations discussed in the previous section, and if so, (b) does the study provide evidence for an MP vector effect that would affect chemical risk in nature. Following Burns and Boxall (2018), the same three weight of evidence categories were distinguished.

#### 6.3.1 Weight of Evidence from In Vivo Laboratory Studies

We reviewed 30 studies providing evidence with respect to the MP vector effect (Table 6.2). None of them explicitly discussed the results with respect to implications for chemical risks as defined by the PEC/PNEC approach (Koelmans et al. 2017). Twenty-one out of thirty studies were evaluated as inconclusive with respect to detection of a chemical vector effect caused by microplastic ingestion. However, there appears to be a trend among studies over time. Earlier studies more often neglect environmentally relevant exposure conditions by focusing on chemical uptake from microplastic alone, resulting in a low (i.e. 'inconclusive') weight of evidence score. Later studies more often include co-exposure from water or also from food or other background particles empirically and/or via modelling, which makes them more relevant for environmental conditions, resulting in a higher weight of evidence (conclusive) score. All of the latter studies, however, conclude that a evidence, also because many of these simulations were done with validated models or backed up with experimental data provided in the same study. Most of the studies concluded that a vector effect is not supported, due to lack of gradient or due to other pathways being more important. Some studies might have overlooked some

|                            |              |              | Not       |  |
|----------------------------|--------------|--------------|-----------|--|
| Study                      | Demonstrated | Inconclusive | supported | Comments   |
| Besseling<br>et al. (2013) |              | X            |           | Measured environmentally relevant<br>exposure of PCBs to <i>A. marina</i> in the<br>lab, including all pathways. Treatments<br>with MP showed higher<br>bioaccumulation. However, aqueous<br>exposure and organism lipids were not<br>measured and could have differed<br>among treatments, which limits<br>interpretation with regard to the MP<br>vector effect  |
| Browne et al. (2013)       |              | X            |           | Exposed initially clean <i>A. marina</i> to<br>MP or sand spiked with phenanthrene,<br>nonylphenol, triclosan or PBDE-47.<br>Co-exposure from water and food was<br>not considered. Considering the<br>experimental design, uptake from<br>water could have occurred as well.<br>Found more uptake from sand than<br>from ingested plastic. This may be<br>explained from the plastic being a<br>more efficient cause for elimination<br>from the test organisms, via particle<br>egestion/defaecation, compared to sand |
| Rochman<br>et al. (2013)   |              | X            |           | Test organisms <i>Oryzias latipes</i> were<br>exposed to MPs that were enriched<br>with environmental contaminants<br>sorbed from San Diego Bay. Uptake<br>from water was not accounted for and<br>not measured. Test organisms were not<br>at equilibrium at the start of the<br>experiment. Not clear which part of<br>uptake was from contaminated food or<br>from water  |
| Chua et al.<br>(2014)      |              | X            |           | Amphipods <i>Allorchestes compressa</i><br>were exposed to PBDEs in the<br>presence or absence of<br>MP. Co-exposure from water and food<br>was not considered, and initially clean<br>organisms were used. Considering the<br>experimental design, uptake from<br>water could have occurred as well.<br>Used unrealistically high plastic<br>concentrations   |

**Table 6.2** Overview of laboratory in vivo studies addressing the role of microplastic ingestion by organisms on bioaccumulation of plastic-associated chemicals

|                            |              |              | Not       |  |
|----------------------------|--------------|--------------|-----------|--|
| Study                      | Demonstrated | Inconclusive | supported | Comments   |
| Avio et al.<br>(2015)      |              | X            |           | Mussels ( <i>Mytilus galloprovincialis</i> )<br>were exposed to MP with and without<br>sorbed pyrene. Uptake from water and<br>natural food was not considered, and<br>initially clean organisms were used.<br>Considering the experimental design,<br>uptake from water could have occurred<br>as well. Used very high plastic<br>concentrations  |
| Wardrop<br>et al. (2016)   |              | X            |           | Rainbow fish ( <i>Melanotaenia fluviatilis</i> )<br>were exposed to MP with and without<br>sorbed PBDEs. Co-exposure from<br>water and food was not considered, and<br>initially clean organisms were used.<br>Considering the experimental design,<br>uptake from water could have occurred<br>as well. Used very high plastic<br>concentrations  |
| Rummel<br>et al. (2016)    |              | X            |           | Studied 'cleaning' of <i>Oncorhynchus</i><br><i>mykiss</i> by microplastic ingestion<br>(reversed vector effect), by feeding<br>PCB-contaminated fish a diet with<br>uncontaminated PE. No observable<br>effect of plastic ingestion on the rate of<br>depuration of PCBs was reported.<br>However, due to the wide confidence<br>intervals in the depuration rate<br>constants, the study had a low<br>sensitivity to detect treatment effects.<br>Hence, the observations may not have<br>been sufficient to make the assumption<br>that the cleaning effect was not present               |
| Besseling<br>et al. (2017) |              |              | X         | Mimicked environmentally relevant<br>exposures of <i>A. marina</i> to PCBs in<br>sediment, MP and water after 6-week<br>equilibration, including all pathways,<br>and assessed ingestion. Aqueous<br>exposure was measured using passive<br>samplers, and organism lipids were<br>measured. Data interpretation was<br>aided by biodynamic modelling based<br>on Koelmans et al. (2013b) (Table 6.4).<br>Bioaccumulation factors did not differ<br>between MP and no-MP treatments,<br>providing evidence for the absence of a<br>vector effect, which was supported by<br>model simulations |

 Table 6.2 (continued)

|                                   |              |              | Not       |   |
|-----------------------------------|--------------|--------------|-----------|---|
| Study                             | Demonstrated | Inconclusive | supported | Comments  |
| Beckingham<br>and Ghosh<br>(2017) |              | X            |           | In vivo study on bioaccumulation of<br>PCBs with <i>Lumbriculus variegatus</i> .<br>Did not consider leaching of PCBs<br>from polypropylene into sediment and<br>water. Did not quantify the relevance<br>of the polypropylene as vector<br>compared to the indirect exposure<br>through desorption to the surrounding<br>media. Did not assess ingestion of<br>microplastic in the worms   |
| Devriese<br>et al. (2017)         |              | X            |           | Norway lobster <i>Nephrops norvegicus</i><br>fed with (short-term spiked) PCB-<br>loaded MPs showed limited additional<br>PCB uptake after 3-week ingestion of<br>PE MP, but not of PS MP. PCBs were<br>MP surface spiked, gelatine food<br>contained PCBs as well, and uptake<br>from water could have occurred as<br>well, impeding differentiation between<br>accumulation from food, water and<br>plastic   |
| Sleight et al.<br>(2017)          |              | X            |           | Tested the hypothesis that MP can act<br>as a vector of chemicals from pelagic<br>to benthic habitats. MP bound<br>chemical bioavailability for zebrafish<br>larvae <i>Danio rerio</i> was assessed via<br>gene expression. Aqueous exposure<br>was not assessed. Sorption equilibrium<br>was assumed in data interpretation, yet<br>not experimentally verified and<br>unlikely given the short (5d) exposure<br>time. MP and chemical concentrations<br>were considerably higher than<br>environmentally relevant<br>concentrations |
| Rochman<br>et al. (2017)          |              | X            |           | Tested how the interaction between<br>microplastics and PCBs could affect a<br>prey species <i>Corbicula fluminea</i> and its<br>predator <i>Acipenser transmontanus</i> .<br>Exposure was to initially clean<br>organisms, via plastic ingestion alone,<br>but not quantified. PCBs could have<br>desorbed to the clean water followed<br>by volatilization of PCBs from<br>microplastics from aeration in the<br>tanks. PCBs were not detected in test<br>animals, so treatment effects on<br>bioaccumulation could not be assessed |

 Table 6.2 (continued)

|                         |              |              | Not       |  |
|-------------------------|--------------|--------------|-----------|--|
| Study                   | Demonstrated | Inconclusive | supported | Comments   |
| Horton et al.<br>(2018) |              | X            |           | Environmentally relevant co-exposure<br>of <i>Daphnia magna</i> to chemicals in MP<br>and water. However, the systems were<br>not at equilibrium partly due to<br>addition of the test organisms. MP<br>concentrations were much higher than<br>in the environment. No effects of MP<br>on chemical toxicity were found  |
| Guven et al.<br>(2018)  |              | X            |           | Tested acute effect of pyrene and MP<br>on swimming and predatory<br>performance of a tropical fish ( <i>Lates</i><br><i>calcarifer</i> ). Not clear if test organisms,<br>MP and pyrene were in equilibrium as<br>effects were recorded between 1 and<br>24 h. fish were not fed, eliminating the<br>realism of this exposure pathway. Not<br>clear how pyrene was measured. The<br>authors confirm that short test duration<br>may have restricted pyrene absorption<br>to the MP  |
| Batel et al.<br>(2018)  |              | X            |           | Tested and confirmed transfer of BaP<br>from MP by simple attachment to clean<br>adult zebrafish ( <i>Danio rerio</i> ) gills and<br>zebrafish embryos. BaP equilibration<br>with MP was for only 24 h. BaP may<br>have desorbed to the water during<br>exposure. Role of waterborne exposure<br>not assessed. No environmentally<br>realistic concentration gradient due to<br>use of clean organisms. Very high MP<br>and BaP levels   |
| Beiras et al.<br>(2018) |              |              | X         | Tested ingestion and contact with PE<br>MP; did not find acute toxicity on<br>marine zooplankton ( <i>Brachionus</i><br><i>plicatilis, Tigriopus fulvus, Acartia</i><br><i>clausi, Mytilus galloprovincialis,</i><br><i>Paracentrotus lividus, Oryzias</i><br><i>melastigma</i> ). Spiking of MP was for 2<br>d only. Contaminated MP was dosed to<br>clean organisms, and no other parallel<br>chemical exposure pathways were<br>taken into account. Despite these<br>conditions of artificially favouring an<br>effect of MP, no effect was found. This<br>means the results can be considered as<br>not supporting the occurrence of an<br>MP vector effect for the species tested |

 Table 6.2 (continued)

|                            |              |              | Not       |  |
|----------------------------|--------------|--------------|-----------|--|
| Study                      | Demonstrated | Inconclusive | supported | Comments   |
| Scopetani<br>et al. (2018) |              |              | X         | Clean <i>Talitrus saltator</i> were fed with<br>uncontaminated fish food mixed with<br>(short term) contaminated MPs, or<br>contaminated <i>Talitrus saltator</i> was fed<br>with contaminated food in combination<br>with clean MP. The first treatment<br>showed a vector effect, whereas the<br>second treatment showed that MP<br>cleaned the organism. Spiking of MP<br>was short term and desorption in the<br>water could have occurred. Given the<br>detection of opposite direction<br>chemical transport, the conclusion of a<br>limited relevance for a vector effect in<br>the environment is considered correct |
| Barboza<br>et al. (2018)   |              | X            |           | Effects of <i>Dicentrarchus labrax</i><br>juveniles upon exposure to MP and<br>HgCl <sub>2</sub> . MP polymer type and<br>associated chemicals not known. Clean<br>fish were used and no food was added,<br>causing the hg speciation and exposure<br>to be different from natural conditions,<br>leaving uncertainty with respect to how<br>MP would make a difference in reality   |
| Rehse et al. (2018)        |              |              | X         | Analysed how the presence of<br>polyamide MP modifies effects of<br>bisphenol A (BPA) on <i>Daphnia magna</i> .<br>BPA exposure was via water only or<br>via water plus ingested MP. $EC_{50}$<br>values were the same, which<br>demonstrates that an MP vector effect<br>did not occur  |
| Beiras and<br>Tato (2019)  |              |              | X         | MP and nonylphenol effects on urchin<br>larvae ( <i>Paracentrotus lividus</i> ).<br>Conditions aimed at maximizing the<br>relevance of the particulate phase for<br>chemical uptake in the test species:<br>Natural particles were not present, and<br>MP loads tested were far above the<br>environmental levels ever found in<br>marine waters. With MP and food<br>present, there were no differences in<br>chemical effect thresholds as compared<br>to when no MPs were present,<br>indicating that no vector effect<br>occurred  |

Table 6.2 (continued)

|                           |              |              | Not       |   |
|---------------------------|--------------|--------------|-----------|---|
| Study                     | Demonstrated | Inconclusive | supported | Comments  |
| Wang et al.<br>(2019)     |              |              | X         | Earthworm <i>Eisenia fetida</i> exposed to<br>PE and PS particles in agricultural soil;<br>PCB-, PAH- and MP-contaminated<br>soil; and PCB/PAH-contaminated soil.<br>Data interpretation was aided by<br>biodynamic modelling based on<br>Koelmans et al. (2013b) (Table 6.4).<br>No MP vector effect was found   |
| Beiras et al.<br>(2019)   |              |              | X         | Sea urchin pluteus and copepod<br>nauplius larvae actively ingest MP<br>particles. MP did not increase<br>accumulation of organic chemicals in<br>sea urchin larvae. MP did not increase<br>the toxicity or 4-n-NP or 4-MBC to<br>zooplankton. PE microplastics did not<br>act as vectors of hydrophobic<br>chemicals to zooplankton  |
| Sørensen<br>et al. (2020) |              |              | X         | Acartia tonsa and Calanus<br>finmarchicus were exposed to<br>ingestible and non-ingestible PE<br>microbeads, spiked with fluoranthene<br>and phenanthrene, which also were<br>present in the water phase. Under this<br>co-exposure scenario, bioaccumulation<br>factors were the same for systems with<br>versus without MP, and for systems<br>with ingestible versus non-ingestible<br>MP, indicating that no MP vector effect<br>occurred |
| Thaysen<br>et al. (2020)  |              | X            |           | Studied the direction of transfer<br>between ingested plastic and biota<br>lipids of brominated flame retardants in<br><i>Larus delawarensis</i> . It was found that<br>the concentration gradients were<br>opposite for different chemicals, and<br>thus would lead to bidirectional<br>transfer dependent on the chemical, yet<br>with transfer from bird to plastic<br>('cleaning') occurring for a higher<br>number of compounds          |

 Table 6.2 (continued)

|                            |              |              | Not       |  |
|----------------------------|--------------|--------------|-----------|--|
| Study                      | Demonstrated | Inconclusive | supported | Comments   |
| Bartonitz<br>et al. (2020) |              | X            |           | <i>Gammarus roeseli</i> were exposed to<br>phenanthrene in water, as well as in the<br>presence of MP or sediment. Due to<br>their high concentrations, MP (and<br>sediment) particles reduced<br>phenanthrene toxicity due to sorption<br>to the particles, and absence of a vector<br>effect was concluded. In nature,<br>however, MP would not reduce<br>aqueous phase concentrations at<br>realistic MP concentrations, rendering<br>the results inconclusive  |
| Xia et al.<br>(2020)       |              | X            |           | Exposed <i>Chlamys farreri</i> to BDE209<br>with versus without presence of PS<br>MP. Bioconcentration factors (BCF)<br>were the same; however, depuration<br>was faster in the presence of MP,<br>demonstrating the 'cleaning' effect.<br>The conditions of MP and BDE209<br>pre-equilibration were not fully clear;<br>co-exposure from the water was not<br>quantified; water was renewed and<br>scallops removed every day, which was<br>not accounted for in the modelling;<br>clean test organisms were used; and no<br>food was present during exposure |
| Coffin et al.<br>(2020)    |              |              | X         | Exposed Atractoscion nobilis to<br>environmentally relevant MP (0.32<br>particles/L) and benzo(a)pyrene-sorbed<br>MP concentrations. Co-exposure via<br>food or water was not included. No<br>effects of the presence of MP were<br>observed in 5-d exposure, indicating<br>that no MP vector effect occurred  |
| Tanaka et al.<br>(2020)    |              | X            |           | Feeding experiment under<br>environmentally relevant conditions, in<br>which PE pellets contaminated with 5<br>additives were fed to <i>Calonectris</i><br><i>leucomelas</i> chicks. The MP vector<br>effect was demonstrated under these<br>experimental conditions. However test<br>animals were clean at start, and the<br>chemicals were not present in the diet,<br>whereas co-exposure via the diet is<br>plausible in nature where most<br>chemicals are ubiquitous   |

 Table 6.2 (continued)

|                        |              |              | Not       |   |
|------------------------|--------------|--------------|-----------|---|
| Study                  | Demonstrated | Inconclusive | supported | Comments  |
| Wang et al.<br>(2020a) |              | X            |           | Earthworm <i>Eisenia fetida</i> exposed to<br>five MP polymer types, spiked with<br>PCB and PAH, over 28 d. Chemical<br>transfer to the worms was observed<br>with dermal exposure generally<br>dominating overexposure via ingested<br>plastic ('vector'). Addition of clean MP<br>reduced transfer ('cleaning'). Data<br>interpretation was aided by biodynamic<br>modelling based on Koelmans et al<br>(2013b) (Table 6.4). It was concluded<br>that MP can act as a source and a sink<br>but that predictions based on short-<br>term non-equilibrium conditions may<br>both be representative of natural<br>conditions |
| Wang et al.<br>(2020b) |              | X            |           | Earthworm <i>Eisenia fetida</i> exposed to<br>PCB-contaminated soil and clean MP<br>(PE of three sizes) or to clean soil and<br>PCB-contaminated MP, for 28 d. Both<br>treatments demonstrated less uptake,<br>i.e. 'cleaning' compared to non-MP<br>controls. Data interpretation was aided<br>by biodynamic modelling based on<br>Koelmans et al (2013b) (Table 6.4).<br>Chemical uptake via MP ingestion was<br>smaller than via the parallel pathways.<br>It was concluded that MP can act as a<br>source and a sink but that any effect in<br>nature would be small due to low MP<br>concentration                     |

Table 6.2(continued)

General approach, type of evidence for an effect on chemical risk and environmental realism are summarized

vector effect is of little relevance under natural conditions (Table 6.2). Four studies explicitly aimed for finding empirical evidence for the 'cleaning' effect by microplastic ingestion (Rummel et al. 2016; Scopetani et al. 2018; Thaysen et al. 2020; Heinrich and Braunbeck 2020), as predicted based on first principles (Gouin et al. 2011; Koelmans et al. 2013b). A cleaning effect was, however, also suggested to occur in the study by Devriese et al. (2017). Browne et al. (2013) found lower chemical body burdens in *A. marina* after chemical uptake via ingested microplastic as compared to uptake via sand, suggesting more efficient 'cleaning' via the plastic as compared to sand. Recently, Xia et al. (2020) reported a statistically significant 30% to 45% faster depuration of BDE-209 from marine scallops in treatments with MP. Bioconcentration factors (BCF) were 6 to 14% higher in the presence of MP, differences which, however, were not statistically significant. An MP 'cleaning' effect was thus demonstrated, in contrast to an MP vector effect.

# 6.3.2 Weight of Evidence from Field Studies

We evaluated six field studies, of which only one was considered to provide conclusive evidence (Herzke et al. 2016), evidence which in this case was not supportive of a chemical vector effect (Table 6.3). The other studies all suffered from the pitfalls of causality, i.e. did not address alternative yet possibly occurring mechanisms that could also explain the observations, such as uptake via water or food.

|                            |              |              | Not       |  |
|----------------------------|--------------|--------------|-----------|--|
| Study                      | Demonstrated | Inconclusive | supported | Comments   |
| Fossi et al.<br>(2012)     |              | X            |           | Effect of microplastic ingestion on<br>bioaccumulation of phthalates was<br>speculated from detection of plastic in<br>plankton samples, and phthalates<br>detected in the same plankton samples<br>and in fin whale ( <i>Balaenoptera physalus</i> ).<br>The plankton to microplastic number<br>concentration ratio was 1600 (Ligurian<br>Sea) to 18000 (Sardinian Sea). Given<br>these ratios and ageing of plastic in the<br>oceans causing chemical desorption and<br>uptake by plankton, uptake via plankton<br>is more likely to occur than via plastic<br>ingestion |
| Gassel<br>et al.<br>(2013) |              | X            |           | Ingestion of plastic was speculated to<br>best explain the detection of nonylphenol<br>in 6 out of 19 fish individuals ( <i>Seriola</i><br><i>lalandi</i> ), given the detection of two<br>plastic particles in 2 out of the 19 fish<br>individuals. Given the data provided, the<br>study shows that plastic may have been<br>the source of the nonylphenol. However,<br>fish (n=19) and plastic (n=2) sample sizes<br>were very low, and parallel pathways<br>may have contributed to the uptake   |
| Tanaka<br>et al.<br>(2013) |              | X            |           | PBDE found in seabirds ( <i>Puffinus</i><br><i>tenuirostris</i> ) were also present in plastic<br>in the stomach, but not in prey,<br>suggesting that the ingested plastic was<br>the source of the PBDEs. However, the<br>prey samples were taken 7 yrs later and<br>>1000km away. The authors<br>acknowledged these caveats  |

 Table 6.3
 Overview of field studies addressing the role of microplastic ingestion by organisms on bioaccumulation of plastic-associated chemicals

...

|                                 |              |              | Not       |   |
|---------------------------------|--------------|--------------|-----------|---|
| Study                           | Demonstrated | Inconclusive | supported | Comments  |
| Rochman<br>et al.<br>(2014)     |              | X            |           | Myctophid sampled at stations with<br>greater plastic densities had larger<br>concentrations of BDEs # 183–209 in<br>their tissues suggesting that these<br>chemicals are indicative of plastic<br>contamination in the marine environment.<br>Plastic was not measured in the fish, and<br>the BDEs might as well have<br>accumulated from water or the plankton<br>diet. No strong conclusion on the role of<br>ingestion was drawn   |
| Herzke<br>et al.<br>(2016)      |              |              | X         | Combined three lines of evidence: (a)<br>correlations among POP concentrations,<br>differences in tissue concentrations of<br>POPs between plastic ingestion<br>subgroups, (b) fugacity calculations and<br>(c) bioaccumulation modelling, to show<br>that MP did not act as a vector of POPs<br>for <i>Fulmarus glacialis</i>  |
| Gassel and<br>Rochman<br>(2019) |              | X            |           | Examined relationships among chemical<br>contaminants and MP in lanternfish<br>(Myctophidae). Lower chlorinated PCBs,<br>higher in gyre fish, correlated with higher<br>modelled plastic density. Data normality<br>and uncertainty in modelled MP densities<br>data potentially affecting significance<br>levels were not taken into account in<br>regression analysis. Exposure pathways<br>other than MP that could also explain the<br>observed differences were not considered |

Table 6.3 (continued)

General approach, type of evidence for an effect on chemical risk and environmental realism are summarized

### 6.3.3 Weight of Evidence from In Vitro Studies

Thirteen in vitro studies reported on the chemical release kinetics from microplastic in the context of the chemical vector effect (Table 6.4). In general, most in vitro studies only reported the amount bioavailable or leached from plastic after exposure in the simulated gut fluid or stomach oil. These bioavailability percentages which are reported generally are not relevant in nature as they also depend on external factors such as fugacities in other compartments in the gut, which would be different in nature. Exchange fluxes with other components in the gut, like food organic matter or micelles, are also usually neglected. Generally, analysis of experiment results with models that describe the data are lacking. This limits the applicability of the findings as they cannot be extrapolated to the actual environment. All studies except two (Mohamed Nor and Koelmans 2019; Kühn et al. 2020) had used a clean gut

|                          |              |              | Not       |  |
|--------------------------|--------------|--------------|-----------|--|
| Study                    | Demonstrated | Inconclusive | supported | Comments   |
| Teuten et al.<br>(2007)  |              | X            |           | Compared desorption rates of sorbed<br>phenanthrene from plastic and<br>sediment in seawater and sodium<br>taurocholate that simulated the gut fluid<br>of <i>Arenicola marina</i> . Model analysis<br>was limited to pseudo first-order rate<br>analysis to determine initial desorption.<br>Considered theoretical calculations<br>using equilibrium partitioning method<br>with different compartments to<br>understand their respective relative<br>contributions. This analysis<br>overestimates the amount bioaccessible<br>to benthic organisms as the<br>environment is more dynamic and it<br>also neglected other pathways |
| Bakir et al.<br>(2014)   |              | X            |           | Investigated the desorption rates of<br>chemicals from PVC and PE under<br>simulated gut conditions. Model<br>analysis was limited as only first-order<br>rate kinetics was considered, neglecting<br>backward sorption and bimodal<br>behaviour. Chemicals were sorbed for a<br>short time, but non-equilibrium<br>desorption kinetics were not<br>considered. Assessment with maximum<br>concentration gradient is not relevant in<br>nature. Other pathways in the gut are<br>neglected   |
| Turner and<br>Lau (2016) |              | X            |           | Evaluated bioaccessibility of elements<br>indicative of halogenated flame<br>retardants in a simulated digestion fluid<br>of seabirds. Model analysis was limited<br>and obtained rate constants using a<br>diffusion-controlled and parabolic<br>model. Assessment with maximum<br>concentration gradient is not relevant in<br>nature. Evaluation of the results in the<br>context of the actual environment is<br>based on total percentage leached from<br>plastic which is not adequate as the gut<br>of the seabirds are already<br>contaminated from other sources  |

**Table 6.4** Overview of laboratory in vitro studies aiming to assess the role of microplasticingestion by organisms on bioaccumulation of plastic-associated chemicals

|                                   |              |              | Not       |   |
|-----------------------------------|--------------|--------------|-----------|---|
| Study                             | Demonstrated | Inconclusive | supported | Comments  |
| Beckingham<br>and Ghosh<br>(2017) |              | X            |           | Compared gut fluid solubilization<br>between polypropylene and other<br>natural and anthropogenic organic<br>particles. The model remained limited<br>and data was analysed assuming that<br>equilibrium was reached for all<br>congeners in the system after 4h.<br>Maximum concentration gradient<br>between contaminated particles and gut<br>fluid was not environmentally relevant   |
| Massos and<br>Turner<br>(2017)    |              | X            |           | Evaluated bioaccessibility of Cd, Pb<br>and Br from beached microplastics in a<br>physiologically based extraction test.<br>Considered only percentage<br>bioaccessible (over total available in<br>the plastic) which does not reflect what<br>happens in nature as the results are<br>only applicable for the case when the<br>organism do not have any accumulated<br>element. Compared bioaccessibility of<br>the elements through plastic against<br>dietary concentrations and did not<br>consider that the percentages may<br>change in the presence of food |
| Turner<br>(2018)                  |              | X            |           | Evaluated bioaccessibility of hazardous<br>elements in simulated digestion fluid of<br>northern fulmar. Model analysis was<br>limited and did not consider accounting<br>for individual exchange fluxes as it<br>uses the pseudo first-order diffusion<br>model. Did not consider the scenario in<br>which the elements have<br>bioaccumulated in the fulmar.<br>Maximum concentration gradients and<br>evaluation of bioaccessibility<br>percentage based on maximum leached<br>(equilibrium concentrations) as in the<br>experiment are not relevant in nature    |
| Lee et al. (2019)                 |              | X            |           | Evaluated relevance of ingested<br>microplastics to overall transfer of<br>chemicals into fish using simulated<br>intestinal fluid and model analysis.<br>Model analysis did not include error<br>estimates and did not reflect<br>experimental results as it was not<br>calibrated. Did not evaluate the results<br>in the context of the actual environment   |

 Table 6.4 (continued)

|                                |              |              | Not       |  |
|--------------------------------|--------------|--------------|-----------|--|
| Study                          | Demonstrated | Inconclusive | supported | Comments   |
| Coffin et al.<br>(2019)        |              | X            |           | Evaluated leaching of plastic additives<br>from commonly ingested plastic items<br>in gut mimic models for fish and<br>seabirds. Used virgin plastic items,<br>maximizing the concentration gradient<br>between plastic and gut fluid, which is<br>not relevant in nature. Single time point<br>statistical analysis of the concentration<br>leaching out from plastic and its effects<br>on estrogenicity only reflects the vector<br>effect under such experimental<br>conditions. Results were not evaluated<br>in the context of other relevant<br>processes                       |
| Guo et al.<br>(2019)           |              | X            |           | Investigated leaching of flame<br>retardants from acrylonitrile-butadiene-<br>styrene (ABS) in simulated avian<br>digestive systems and the effect of<br>co-ingesting sediment. Did not<br>consider further evaluation of results<br>with models or calculations with<br>chemical characteristics ( $K_{ow}$ ). Results<br>indicated chemicals transferred from<br>plastic to sediment. However, the study<br>did not consider sorption capacities of<br>the components in the system and its<br>exchange fluxes   |
| Martin and<br>Turner<br>(2019) |              | X            |           | Evaluated the mobilization of cadmium<br>under simulated digestive conditions<br>over 6h. Model was limited and did not<br>consider the other phases and exchange<br>fluxes. Applicability of the rate<br>constants obtained by fitting a<br>second-order diffusion model to the<br>experimental data was not discussed.<br>Evaluation of environmental<br>implication is not relevant in nature as<br>there is no clean sediment in nature.<br>Sediment used in experiment was<br>collected from a protected and<br>unpolluted location which is not<br>representative of other areas |

Table 6.4 (continued)

|  |              |              | Not       |   |
|--|--------------|--------------|-----------|---|
| Study                                    | Demonstrated | Inconclusive | supported | Comments  |
| Mohamed<br>Nor and<br>Koelmans<br>(2019) |              |              | X         | Evaluated chemicals exchange of PCBs<br>in gut fluid mimic systems considering<br>different scenarios representing<br>different fugacity levels between plastic<br>and organism. Considered all exchange<br>fluxes in the system and bimodal<br>behaviour in model analysis, showing<br>error estimates for parameters.<br>Considered exchange fluxes with other<br>components in the gut such as food.<br>Discussed bioavailability from ingested<br>plastic to organisms under different<br>scenarios                                     |
| Heinrich and<br>Braunbeck<br>(2020)      |              | X            |           | Studied the effect of PE MP addition to<br>rainbow trout RTL-W1 cells dosed<br>with 7-ethoxyresorufin-O-deethylase<br>(EROD) inducers by addition. The<br>addition of MP reduced EROD activity.<br>The authors concluded that the<br>presence of MP can reduce the amount<br>of bioavailable pollutants in situ<br>('cleaning' effect) but that it remains<br>unclear to what extent this mechanism<br>occurs under natural conditions  |
| Kühn et al.<br>(2020)                    |              | X            |           | Observed leaching of 5 (out of 15)<br>chemicals to stomach oil sampled from<br>fulmars. Results of different<br>experiments were said to be<br>inconsistent. Concentrations in the oil<br>could have decreased due to<br>biodegradation, increasing the gradient<br>for transfer. Biomagnification from<br>regular food was not addressed.<br>Chemical transfer from the oil to the<br>plastic was not considered. It was<br>acknowledged that the experimental<br>data should not be compared with<br>models simulating natural conditions |

 Table 6.4 (continued)

General approach, type of evidence and environmental realism are summarized

system, which is unrealistic. This may result in an overestimation of the amount leached from plastic. Kühn et al. (2020) used natural contaminated stomach oil and indeed only found limited transfer for five and no transfer for the other ten chemicals studied. Only few studies relate experimental observations with the chemical and sorbent characteristics. Most studies did not provide error estimates or significance levels for their parameter estimations. Due to these limitations, 12 out of 13 studies were evaluated as inconclusive. The one study providing conclusive evidence demonstrated that the occurrence of a vector effect was context-dependent

(Mohamed Nor and Koelmans 2019). They showed that the boundary conditions, i.e. where either the MP or the food was initially contaminated, or both, determined whether transfer occurred and in which direction.

# 6.3.4 Weight of Evidence from Modelling Studies

Twelve studies used models to investigate the microplastic vector effect (Table 6.5). Most of them were explicitly applied to simulate exposure to plastic-associated chemicals under natural conditions, taking multiple exposure pathways into account, or to simulate realistic experiments that also had multiple exposure pathways covered in their experimental design. These studies generally provided conclusive

|                               |              |              | Not       |   |
|-------------------------------|--------------|--------------|-----------|---|
| Study                         | Demonstrated | Inconclusive | supported | Comments  |
| Teuten<br>et al.<br>(2007)    |              | Xª           |           | An equilibrium model scenario predicted<br>a cleaning effect of plastics in reducing<br>contaminant concentrations in benthic<br>organisms. Another scenario predicted<br>enrichment of chemical concentrations<br>on plastic in the surface microlayer<br>(SML) leading to higher exposure to<br>benthic organisms. The acclaimed SML<br>enrichment, however, has been argued to<br>be based on a misinterpretation, as<br>discussed in Koelmans (2015).<br>Desorption during microplastic settling<br>and burial was not accounted for. Uptake<br>by sediment or food ingestion was not<br>considered |
| Gouin<br>et al.<br>(2011)     |              |              | Xª        | Considered all known accumulation<br>pathways in order to quantitatively assess<br>the relative importance of plastic<br>ingestion to total bioaccumulation. A<br>worst case was considered by assuming<br>concentrations in plastic and tissue to be<br>at steady state. Provided mechanistic<br>evidence based on first principles  |
| Koelmans<br>et al.<br>(2013b) |              |              | X         | Considered all known accumulation<br>pathways in order to quantitatively assess<br>the relative importance of plastic<br>ingestion to total bioaccumulation for a<br>previously published dataset. Provided<br>mechanistic evidence based on first<br>principles, as well as causal evidence on<br>the treatment level by validation with<br>empirical data   |

**Table 6.5** Overview of studies that used simulation models to address the role of microplasticingestion by organisms on bioaccumulation of plastic-associated chemicals

|                               | 1            | 1            |     |   |
|-------------------------------|--------------|--------------|-----|---|
| Study                         | Demonstrated | Inconclusive | Not | Comments  |
| Koelmans<br>et al.<br>(2014)  | Demonstrated | Inconclusive | Xª  | Compared bioaccumulation due to plastic<br>ingestion only, with total observed<br>bioaccumulation in the field. Provided<br>mechanistic evidence based on first<br>principles. The model was validated<br>elsewhere (Koelmans et al. 2013b, 2016)   |
| Bakir et al.<br>(2016)        |              |              | X   | Considered all known accumulation<br>pathways in order to quantitatively assess<br>the relative importance of plastic<br>ingestion to total bioaccumulation.<br>Neglected backward sorption. The 50%<br>scenario can be considered unrealistic or<br>worst case. Provided mechanistic<br>evidence based on first principles   |
| Koelmans<br>et al.<br>(2016)  |              |              | Xª  | Simulated a series of published<br>experiments using spiked plastic and<br>clean organisms. Provided mechanistic<br>model validation based on three lines of<br>evidence: (a) intrapolymer diffusion, (b)<br>in vitro desorption kinetic data to<br>artificial gut fluids and (c) evaluation<br>against experimental data from three<br>published datasets. Simulations<br>representing natural exposure conditions<br>demonstrated the vector effect to be<br>negligible |
| Herzke<br>et al.<br>(2016)    |              |              | Х   | Modelled chemical fluxes of microplastic<br>ingested by seabirds, accounting for all<br>uptake pathways. Revealed that plastic<br>was more likely to act as a passive<br>sampler than as a vector for chemicals   |
| Rochman<br>et al.<br>(2017)   |              | X            |     | Used the model developed by Koelmans<br>et al. (2013b) to simulate chemical<br>concentrations from a dietary uptake<br>experiment, which however could not be<br>measured due to detection limit<br>problems. Only uptake via plastic was<br>addressed; uptake from food or water<br>was not accounted for  |
| Besseling<br>et al.<br>(2017) |              |              | X   | Modelled experimental data on chemical<br>uptake by lugworms accounting for all<br>pathways (plastic water, food). Aqueous<br>exposure was assessed using passive<br>samplers. The ingestion vector effect was<br>demonstrated to be irrelevant   |

Table 6.5 (continued)

|                                      |              |              | Not       |   |
|--------------------------------------|--------------|--------------|-----------|---|
| Study                                | Demonstrated | Inconclusive | supported | Comments  |
| Diepens<br>and<br>Koelmans<br>(2018) |              | X (PAHs)     | X (POP)   | Reported a food web model<br>(MICROWEB) based on the model by<br>Koelmans et al. (2013b), which has been<br>validated for POPs. For POPs no vector<br>effect was predicted across all trophic<br>levels of the food web (evidence for<br>'non-supported'). For degrading<br>compounds, a vector effect was<br>predicted, which, however, has not yet<br>been experimentally validated and<br>therefore was rated 'inconclusive'   |
| Lee et al.<br>(2019)                 |              |              | X         | Considered all known pathways to assess<br>the contribution of plastic ingestion to<br>chemical bioaccumulation with an<br>uncertainty analysis using Monte-Carlo<br>simulation. Did not use results from<br>in vitro experiments in model to obtain a<br>more refined model  |
| Wang et al. (2019)                   |              |              | X         | Modelled experimental data on chemical<br>uptake by earthworms ( <i>Eisenia fetida</i> )<br>accounting for all pathways (plastic,<br>water, food), using the model by<br>Koelmans et al. (2013b). Concentrations<br>in soil (food) and water were measured.<br>Modelled and empirical data agreed well<br>and no vector effect was found  |
| Wang et al.<br>(2020a)               |              | X            |           | Modelled experimental data on chemical<br>uptake by earthworms ( <i>Eisenia fetida</i> )<br>accounting for all pathways (plastic,<br>water, food), using the model by<br>Koelmans et al. (2013b) for contaminated<br>plastic – clean worm scenarios ('vector<br>effect'). Chemical transfer to the worms<br>was observed with dermal exposure<br>generally dominating overexposure<br>through MP ingestion. A minor vector<br>effect thus was found, but it remained<br>unclear to what extent this would apply<br>under natural conditions |

 Table 6.5 (continued)

|                        |              |              | Not       |   |
|------------------------|--------------|--------------|-----------|---|
| Study                  | Demonstrated | Inconclusive | supported | Comments  |
| Wang et al.<br>(2020b) |              | X            |           | Modelled experimental data on chemical<br>uptake by earthworms ( <i>Eisenia fetida</i> )<br>accounting for all pathways (plastic,<br>water, food), using the model by<br>Koelmans et al. (2013b) for a<br>contaminated plastic – clean worm<br>scenario ('vector effect'), as well as the<br>opposite ('cleaning effect'). Both effects<br>were found implying that the vector<br>effect is context-dependent |

Table 6.5 (continued)

<sup>a</sup>Similarly assessed earlier by Burns and Boxall (2018)

General approach, type of evidence for an effect on chemical risk and environmental realism are summarized

aspects, such as release kinetics, gut retention times or reversible (backward) sorption, aspects that however would lead to an overestimation of the importance of the vector effect. Studies that considered such 'worst-case' conditions but still concluded that a vector effect was minor were evaluated as conclusive. Diepens and Koelmans (2018) provided theory suggesting that chemicals present at higher than equilibrium concentrations, like additives, in some cases can (over-)compensate for the simultaneously occurring cleaning effect, leading to a net MP vector effect if plastic mass concentrations are high. Furthermore, they calculated that degradable compounds such as PAH may also be prone to a vector effect due to the chemicals being less bioavailable for metabolization during gut passage. It must be noted that the credibility of their model applies to all scenarios modelled (equilibrium and 'under'-equilibrium) and that the scenarios that represent equilibrium conditions for persistent (i.e. non-metabolizable) chemicals are more likely to be relevant (Koelmans et al. 2016; De Frond et al. 2019).

# 6.4 Risk Assessment of Plastic-Associated Chemicals: A Case Study Illustrating the Relevance of the MP Vector Effect for Risks of Plastic-Associated Chemicals in San Diego Bay

The previous sections have shown that studies addressing the MP vector effect often did not put their results in an environmentally realistic context or did not address results in the context of chemical risks. Here, the notion 'risk' is not meant as probability, hazard, harm or threat but as the extent to which exposure chemical concentrations exceed a known effect threshold for the species for which the risk is then assessed. We use this definition because it is objective and quantitative and because it is the metric that is used for risk assessment of chemicals in regulatory frameworks (Koelmans et al. 2017). As mentioned, this context of actual risks is relevant, because if a detected MP vector effect would not affect risks of the chemicals, then it might be less urgent to study it and there might be less reason for concern.

Description of the Case For the case study, we use the pioneering laboratory experiment by Rochman et al. (2013) where Oryzias latipes (Japanese medaka) were exposed to MP loaded with HOCs sorbed from San Diego Bay seawater. Note that this Asian species was used as test organisms and was not claimed to live in San Diego Bay. For the case study, we use the PCB data from their study and calculate back what medaka would have been exposed to, when they would actually reside in San Diego Bay. In the laboratory study, the observed increase in PCB bioaccumulation by medaka was ascribed to the ingestion of MP. The MP was equilibrated in San Diego Bay, which yielded field relevant PCB concentrations on the plastic and which also contributed to the environmental relevance of the study. The fact that the MPs acquired PCBs from the seawater implies that there were PCBs in the water. Therefore, in the bay, medaka would have been exposed to plastic-associated PCBs as well as to the same PCBs dissolved in the water. Additionally, fish would also be exposed to PCBs in food (prey), which, similar to the MPs, would have absorbed or bioconcentrated PCBs from the ambient water. In the laboratory experiment, however, aqueous phase concentrations were kept at zero. Furthermore, in the experiment, fish was fed PCB contaminated cod liver oil. PCB concentrations were known, but these were not equal to concentrations that would be present in natural food at equilibrium in the bay. Furthermore, in the laboratory experiment, fish were already contaminated with PCBs, but at lower than equilibrium concentrations, because uptake was observed, in the controls as well as in the plastic treatments. In contrast, in the bay, chemical concentrations in small fish like medaka would have been at steady state.

of the **Contribution** of Plastic in the Calculation Laboratory *Experiment* Koelmans et al. (2016) modelled the experiment published by Rochman et al. (2013) with aqueous concentration set to zero and ingestion rate and concentrations in food set at the values reported in the study. This model framework has been developed, applied and validated (Table 6.5) in a series of studies (Koelmans et al. 2013a, b, 2014, 2016; Koelmans 2015; Rochman et al. 2017; Wang et al. 2019, 2020a, b). A similar model has been applied by Bakir et al. (2016) to assess the relative contribution of chemical uptake pathways for marine worms, fish and seabirds. This modelling shows that in the experiment (Rochman et al. 2013), the contribution of the ingested MP to bioaccumulation by medaka in the experiment can be estimated to range from 3% to 100%, depending on the PCB congener (Table 6.6).

|       | PCB18 | PCB28 | PCB52 | PCB44 | PCB101 | PCB123 | PCB118 | PCB153 | PCB138 | PCB187 |
|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| Water | 0.0   | 0.0   | 0.0   | 0.0   | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    |
| Food  | 62.7  | 0.0   | 28.6  | 0.0   | 22.4   | 96.8   | 38.3   | 40.1   | 40.1   | 58.2   |
| MP    | 37.3  | 100   | 71.4  | 100   | 77.6   | 3.22   | 61.7   | 59.9   | 59.9   | 41.8   |

**Table 6.6** Percentage uptake from water, food and plastic for PCBs in the lab experiment by Rochman et al. (2013), modelled according to data provided

*Calculation of the Contribution of Plastic Occurring Under Natural Conditions in San Diego Bay* Using the model with the same parameter values, it can be calculated what the contribution of MP ingestion would be, if (a) PCB exposure from water is included as it would occur in San Diego Bay, if (b) exposure from natural food is included as it would occur in the bay and if (c) the background concentration in fish from the bay would be taken into account. Altogether, this calculation thus demonstrates what the contribution of MP ingestion would have been under natural, non-laboratory conditions. This calculation uses the simple mass balance principle as follows (Fig. 1):

$$UPTAKE BY FISH(t) =$$

$$(A)UPTAKE FROM WATER +$$

$$(B)UPTAKE FROM FOOD +$$

$$(C)UPTAKE FROM PLASTIC -$$

$$(D)LOSSES FROM DEPURATION, EGESTION, DEFAECATION.$$

$$(6.1)$$

In mathematical form with terms A, B, C and D in the same order (Koelmans et al. 2013a, b, 2014, 2016, Koelmans 2015):

$$\frac{dC_{B,t}}{dt} = k_{derm}C_{W} + IR \times S_{FOOD}a_{FOOD}C_{FOOD} + IR \times S_{PL}C_{PLR,t} - k_{loss}C_{B,t}$$
(6.2)

The fraction uptake from plastic is calculated as term C divided by the sum of the uptake terms A, B and C.

For uptake from water (term A), the absorption rate constant is needed, which is taken from Hendriks et al. (2001). The chemical concentration in the water ( $C_W$ ) is obtained from the chemical concentrations on the plastic ( $C_{PL}$ ) as reported by Rochman et al. (2013) and the equilibrium partition coefficient for PE,  $K_{PE}$  (Lohmann 2011), according to  $C_W = C_{PL}/K_{PE}$ .

Fish food (term B) can be considered as organic matter. The PCB concentration in the food at equilibrium as it would occur in the bay can be calculated from  $C_W$ calculated above and an organic matter (OM) partition coefficient  $K_{OM}$ , which can be calculated from the octanol-water partition coefficient  $K_{OW}$  (log $K_{OM}$ =Log $K_{OW}$ -0.48, (Seth et al. 1999)). The contribution from ingested MP (term C) is modelled as described in Koelmans et al. (2016). This calculation accounts for partition coefficients to plastic, residence times for plastic in the gut and net absorption (leaching and readsorption) rates inside the organism. The fraction of plastic in the ingested material was kept at 10%, in accordance to the study (Rochman et al. 2013).

Loss from depuration, egestion and defaecation (term D) is also modelled as described in Koelmans et al. (2016). However, it does not play a role in the calculation of the percentage of chemical uptake from microplastic ingestion.

The background concentration in the hypothetical San Diego Bay medaka can be calculated from the same  $C_w$  (above) and the lipid normalized bioconcentration factor, which can be equated to  $K_{ow}$ . This assumes that these lipids are at equilibrium with water. In reality these lipids may have a higher than equilibrium concentration due to biomagnification. This implies that the present calculation would overestimate the relative contribution of plastic, because the actual fugacity gradient between the fish lipids and ingested plastics is not taken into account.

The calculation shows that in this environmentally realistic scenario, food intake is the major PCB uptake pathway, and ingestion of MP would make a negligible contribution to uptake by medaka (Table 6.7).

*Validity of Assumptions and Calculations* The concentrations in MP were taken from Rochman et al. (2017) and were measured after a 3-month exposure in San Diego Bay, which implies that the estimates of the aqueous concentrations in the bay ( $C_w$ ) are quite accurate, given that PCB sorption to small MP reaches equilibrium in 3 months (De Frond et al. 2019; Mohamed Nor and Koelmans 2019). Sorption to small organic matter particles, phytoplankton or zooplankton is also within days (Koelmans et al. 1993, 1997), which implies that using a  $K_{OM}$  for estimating concentrations in food as they would occur in the bay is defensible. Given the small sizes of the early life stages of fish like medaka, they are subjected to fast equilibration kinetics while they grow from egg and larvae size to adult life stage, which implies PCB body burden are maintained at steady state.

**Discussion** The calculation suggests that the experiment by Rochman et al. (2013) occurred at non steady-state conditions due to zero concentration in the water and lower than equilibrium (compared to the plastic) concentration in the food. The fugacity was thus higher in the plastic than in food and especially water. Natural systems strive towards chemical equilibrium and steady state and reach such a state usually because of chemical transfer rates that are sufficiently fast compared to residence times of particles and animals in nature. The experimental conditions as in Rochman et al. (2013) were supplemented to align with the exposure scenario we

|       | PCB18 | PCB28 | PCB52 | PCB44 | PCB101 | PCB123 | PCB118 | PCB153 | PCB138 | PCB187 |
|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
| Water | 4.37  | 3.16  | 1.19  | 1.19  | 0.48   | 0.39   | 0.39   | 0.19   | 0.19   | 0.11   |
| Food  | 95.5  | 96.7  | 98.7  | 98.7  | 99.4   | 99.5   | 99.5   | 99.6   | 99.6   | 99.7   |
| MP    | 0.097 | 0.10  | 0.12  | 0.12  | 0.14   | 0.15   | 0.15   | 0.16   | 0.16   | 0.17   |

Table 6.7 Percentage uptake from water, food and plastic for PCBs in San Diego Bay

have chosen as relevant to the environment, by adding exposure from water, assuming sorption equilibrium between plastic and water and setting the fugacity in the food and fish lipids to the same value as the fugacity in the plastic exposed in the bay. This recalculation to such an equilibrium setting shows that the contribution to chemical transfer by ingestion of MP becomes very small. There is some uncertainty in the coefficients used, and newly added MP may, for some time, have a higher than equilibrium fugacity compared to the other media present. Still, even if concentrations in plastic would be one or two orders of magnitude higher and chemicals inside the microplastic would not re-partition to water and/or food, MP would still be the minor source. The present calculation is conservative because (a) biomagnification, which attenuates the gradient for uptake between ingested plastic and animal tissue (Diepens and Koelmans 2018), was not taken into account and (b) the fraction of plastic in the food was 10%, which is a rather high value for many habitats. Finally, the model-aided recalculation illustrates how information from experimental studies can inform questions related to other systems, for instance, environmentally relevant natural systems.

*Implications for Chemical Risks* Here we provide an example of the reasoning that would be needed to assess the implication of the MP vector effect on risk, again with San Diego Bay as an example. First of all, the results (Table 6.7) demonstrate that ingestion of MP contributes a negligible fraction to total PCB bioaccumulation. In other words, there is no MP vector effect in San Diego Bay, and thus there is no implication for risk. If however, ingestion of MP would have contributed for 50%, 90% or 99% of all uptake, exposure could be increased by, for instance, factors of 2, 10 and 100, respectively. Whether such MP vector effects would affect chemical risk then depends only on whether such increases would bring the risk characterization ratio (MEC/PNEC) to a value larger than 1. The sum  $\Sigma$ PCB concentration calculated for San Diego Bay based on the measured concentrations on MP is  $1.8 \times 10^{-5}$  $\mu$ g/L, whereas the EPA water quality standard for  $\Sigma$ PCB is 0.03  $\mu$ g/L, which is 1600 times higher than the  $\Sigma$ PCB aqueous concentration. This implies that even if MP ingestion would increase exposure by a factor of 100, MP ingestion would not have implications for the risks of the chemicals. Two disclaimers need to be mentioned. First, there are other HOCs in the water, such as PAHs and PBDEs, which could potentially change this chemical risk assessment. PAH do not readily bioaccumulate as they are metabolized by the fish but may still lead to higher exposure via an MP vector effect (Diepens and Koelmans 2018). PBDEs have a similar concentration and behaviour pattern as PCBs and thus would roughly double the  $\Sigma$ HOC risk profile, yet still rendering it negligible. Second, the specific outcome of this first case study is meant as an example of how model simulation can complement experimental data to render experimental studies more relevant for natural conditions. However, that does not imply that the conclusion for San Diego Bay can be generalized, as it is only the first analysis of this kind. Although the situation may be similar for many locations, it cannot be precluded that implications for risks are absent on all possible locations or in the future when MP concentrations increase.

# 6.5 Mitigation of Microplastic and Plastic-Associated Chemicals

The problem of global pollution with plastic debris has increased awareness within the public, the scientific community as well as policy makers. This has led to a wide debate on solutions for this problem. General consensus is that there is no single 'silver bullet' solution but that when we are to reduce the presence of plastic debris in the environment, this has to be achieved from a combination of measures (Alexy et al. 2019). These include reducing emissions, reducing littering, use of different and 'safe by design' types of polymers and products, close the waste cycle, recycling and cleaning (SAPEA 2019).

The present article addresses implications of chemicals associated with microplastic. This renders the question to what extent remediation approaches for MP-associated chemicals exist. Recently, De Frond et al. (2019) coined the idea of 'microplastic mitigation is chemical mitigation'. They argue that due to their sorptive properties, plastics have been suggested as a management tool to clean chemicals from the water column (Zhu et al. 2011; Tomei et al. 2015). Removing plastics that contain chemicals from the marine environment removes these chemicals, reducing exposure to wildlife and decreasing potential harm. The mitigation approach would be more efficient for locations with higher microplastic densities, i.e. more on beaches and in coastal zones than in oceanic gyres.

This approach is likely to be useful, especially for locations with high plastic pollution. There also may be limitations, related to how the chemicals are actually distributed in the system to be remediated. For efficient environmental remediation of chemicals, it needs to be assessed where the chemicals are. For instance, De Frond et al. (2019) expressed concern for chemicals in the marine environment, which thus renders the question where these chemicals actually reside under the specific conditions of marine systems. In their paper, they assume chemical equilibrium, thereby following the basic principles of environmental chemistry with respect to chemical kinetics and thermodynamics. When we apply these to the marine environment, we would consider microplastic particles as passive samplers, polymer particles being in equilibrium with the ambient water (Seidensticker et al. 2019). Consequently, aqueous phase concentrations can be calculated, because the plastic to water partition coefficients are known. Given the aqueous phase concentrations and the size of a marine compartment (i.e. coastal zone or oceanic gyre subsystem), the mass of chemical in that compartment can be assessed. Such calculations have been provided by Koelmans et al. (2016) who calculated that a negligible  $2 \times 10^{-4}$  % of chemical mass in the global ocean would be associated with plastic. De Frond et al. (2019) reported concentrations of PCBs on plastic of up to 757 ng/g, at a plastic density up to a maximum 249 g/m<sup>2</sup>, albeit measured at different locations. Still, as a worst-case calculation, if we use 50% of these values as a proxy for the means of the actual concentrations and assume total suspended solids (TSS) concentrations of 10 mg/L and a mean PCB partition coefficient of 10<sup>5</sup> L/kg, then a water column of 60 m below such a square metre with 249 g of MP particles

would hold the same mass of PCBs (i.e. the sum of the mass of PCBs sorbed on TSS and the mass dissolved in water) as all of these MP particles at the surface. Plastic removal would thus only remove a small fraction of the chemicals in marine systems. In coastal areas, there is more chemical mass on plastic than there is on plastic in the open ocean; however, nutrient loads are also higher because the anthropogenic activities leading to plastic pollution are the same ones that cause nutrient and organic pollution (Strokal et al. 2019). Therefore, the chemical fractions on plastic are even lower in coastal areas, which therefore limits the effectiveness of chemical mitigation by cleaning up (micro-)plastic.

#### 6.6 General Discussion and Conclusion

The evaluation of 61 studies addressing the microplastic vector effect revealed that the evidence for the occurrence of the effect in nature is generally weak. This is because many studies remained inconclusive with respect to the hypothesized effect. Other studies were more conclusive; however, they generally provided evidence for the absence of a vector effect.

We suggested using a risk assessment perspective in order to provide context and meaning in case a vector effect would be detected. However, none of the reviewed studies provided such a risk assessment, that is, a quantification of to what extent the occurrence of a vector effect causes chemical exposure to exceed safety effect thresholds. As a proof of concept, we provided an analysis of existing data for San Diego Bay as a case study, where no implications for risks were predicted.

Finally, we emphasize that realizing that the weight of evidence for an MP vector effect to occur in the environment may be low is not the same as proving there is no risk from ingestion of plastic (e.g. Wardman et al. 2020). It has been demonstrated that ingestion of microplastic can lead to physical effects, effects that translate into ecological risks as soon as threshold effect concentrations are exceeded. Theoretically, plastic ingestion can also increase chemical risks. It is just a matter of plastic and chemical concentration levels that in the end determine to what extent chemical exposure is increased as compared to a chemically contaminated environment without plastic particles present. However, at present, the concentrations of microplastic generally seem too low and the alternative exposure pathways too important to cause ingestion of microplastic to make a difference with respect to the risks of chemicals.

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