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Toxicity of microplastics and natural particles in the freshwater dipteran *Chironomus riparius*: Same same but different?

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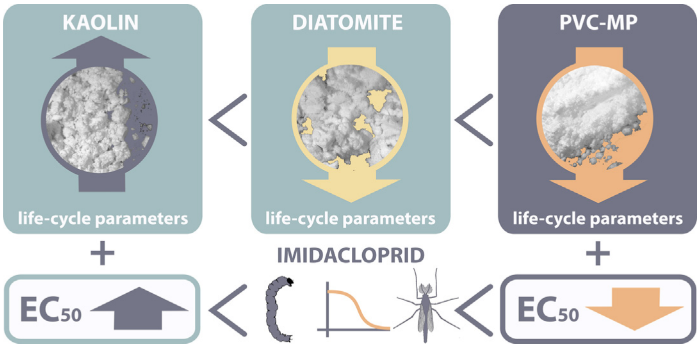
HIGHLIGHTS

- Polyvinyl chloride microplastics (PVC-MP), kaolin and diatomite affected chironomids in a concentration-dependent manner.
- Kaolin positively affected the emergence of chironomids.
- PVC-MP and diatomite negatively affected the emergence and weight of chironomids.
- PVC-MP was more toxic than natural particulate materials.
- PVC-MP exacerbated the toxicity of imidacloprid by acting as an additional stressor.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MP) are contaminants of emerging concern in aquatic ecosystems. While the number of studies is rapidly increasing, a comparison of the toxicity of MP and natural particulate matter is largely missing. In addition, research focusses on the impacts of hydrophobic chemicals sorbed to plastics. However, the interactive effects of MP and hydrophilic, dissolved chemicals remain largely unknown. Therefore, we conducted chronic toxicity studies with larvae of the freshwater dipteran *Chironomus riparius* exposed to unplasticised polyvinyl chloride MP (PVC-MP) as well as kaolin and diatomite as reference materials for 28 days. In addition, we investigated the effects of particles in combination with the neonicotinoid imidacloprid in a multiple-stressor experiment. High concentrations of kaolin positively affected the chironomids. In contrast, exposure to diatomite and PVC-MP reduced the emergence and mass of *C. riparius*. Likewise, the toxicity of imidacloprid was enhanced in the presence of PVC-MP and slightly decreased in the co-exposure with kaolin. Overall, parallel experiments and chemical analysis indicate that the toxicity of PVC-MP was not caused by leached or sorbed chemicals. Our study demonstrates that PVC-MP induce more severe effects than both natural particulate materials. However, the latter are not benign *per se*, as the case of diatomite highlights. Considering the high, environmentally irrelevant concentrations needed to induce adverse effects, *C. riparius* is insensitive to exposures to PVC-MP.

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1. Introduction

The continuous release and break down of large plastic debris as well as direct emissions result in an accumulation of microscopic plastics, so-called microplastics (MP), in aquatic environments (Andrady, 2017; Conkle et al., 2018; Geyer et al., 2017; Hartmann et al., 2019). While a large number of studies address the abundance of MPs in aquatic systems, studies on their toxicity are less prevalent, especially in a freshwater context (Blettler et al., 2018). So far, toxicity studies cover a variety of outcomes, including null effects, reduced growth and reproduction, elevated mortality and inflammatory response (e.g., reviewed in Foley et al., 2018). MPs may act as vectors, transferring additives and sorbed pollutants to biota or affect the impacts of pollutants by altering their bioavailability (Wang et al., 2018). In theory, hydrophobic organic chemicals (high log K_{OW}) preferably sorb to MPs or other particulate matter, while less hydrophobic substances (low log K_{OW}) rather remain dissolved (Wang et al., 2018). Whereas most studies address the role of MP to act as a vector by using hydrophobic chemicals, effects by co-exposures to hydrophilic ones are scarce and mostly overlooked in MP research (Horton et al. 2018; Triebskorn et al., 2019).

In general, the toxicity of MPs depends on their physicochemical properties (material, size, shape etc.), the pollutant (e.g., log K_{OW}), and the species. Accordingly, generalizations with regards to the environmental risk of MPs is challenging as they represent a very diverse group of stressors (Lambert et al., 2017; Scherer et al., 2017b). Thus, it is unsurprising that the relevance of MPs as anthropogenic contaminants is discussed controversially (Burton, 2017; Kramm et al., 2018). One key question to assess the environmental risks of MPs is their toxicological impact compared with natural particulate matter (Backhaus and Wagner, 2018). Numerous studies have shown that suspended solids affect a range of species depending on the material and the particle size (e.g., reviewed in Bilotta and Brazier, 2008) The same is true for the composition of sediments. For example, organic carbon content, material composition as well as grain-size distribution of sediments has been shown to affect growth and survival of benthic invertebrates (Ankley et al., 1994; Bisthoven et al., 1998; Ristola et al., 1999). The presence of particulate matter in the water phase or in sediments also modulates the exposure to chemicals and their toxicity (Fleming et al., 1998; Landrum and Faust, 1994; Zhang et al., 2018). While this is relevant in an MP context, most toxicity studies do not compare the impacts of natural particles to that of MPs (Burns and Boxall, 2018; Connors et al., 2017; Scherer et al., 2017b). Accordingly, the context is missing to benchmark the impacts of MPs in environments in which natural particulate matter is abundant.

Thus, the aim of this study is to address the outlined knowledge gaps: First, we compare the toxicity of MPs to that of natural particulate matter (NPM) present in sediments or suspended in the water phase. Second, we investigate changes in toxicity of a hydrophilic chemical in the presence of MPs and NPM. Focussing on freshwater systems, we exposed the benthic deposit feeding larvae of *Chironomus riparius* to unplasticized polyvinyl chloride (PVC-MP) as well as kaolin and diatomite as NPM. We selected kaolin and diatomite as references materials based on the following reasoning: (1) Both are naturally occurring sediment components frequently applied in toxicity experiments (OECD, 2004). (2) Due to their high densities, they settle rapidly and are thus bioavailable for *C. riparius*. (3) Their physicochemical properties and associated effects are well-characterized (e.g., Hadjar et al., 2008; Murray, 2006; Nattrass et al., 2015; OECD, 2004). We have previously shown that *C. riparius* readily ingests MPs and, consequently, represent an ideal model to study the effect of dense particulate materials on benthic organisms (Scherer et al., 2017a).

In 28 d chronic toxicity experiments, we investigated the effects of the three fine particulate materials (FPM) kaolin, diatomite and PVC-MP on the emergence, development and mass of chironomids. Moreover, we evaluated the impact of kaolin and PVC-MP in chironomids co-exposed to the neonicotinoid pesticide imidacloprid in a multiple-stressor experiment. Imidacloprid is hydrophilic and, thus, sorption to FPM will be negligible. Therefore, an increased toxicity of imidacloprid is expected to be an additional stress induced by FPM rather than an effect of a higher bioavailability.

2. Methods

Aquatic larvae of the diptera *C. riparius* were exposed to different concentrations of PVC-MP and NPM with and without co-exposure to the neonicotinoid imidacloprid (CAS: 138261-41-3, Sigma-Aldrich) in 28 d, chronic toxicity experiments. *C. riparius* larvae were obtained from our in-house culture by transferring egg ropes into petri dishes containing Elendt M4 medium (OECD, 2004). The egg ropes were incubated at 20 °C and a light:dark cycle of 16:8 h. Hatched larvae were monitored daily to collect chironomids < 24 h for the experiments.

PVC powder with particle sizes < 50 μm (PyroPowders, Erfurt, Germany) was used. To discriminate general particle and MP-specific toxicity, kaolin (CAS: 1332-58-7, Sigma-Aldrich) and/or diatomite powders (CAS: 91053-39-3, Sigma-Aldrich) were used as NPM reference. The size distributions of the three FPM were analysed using a Multisizer 3 (Coulter Counter, Figure 1, S1).

2.1. Chronic exposures studies with Chironomus riparius

The experiments were performed in accordance with the OECD guideline 218 (OECD, 2004) with a modified sediment composition: While the guideline suggests a high proportion of FPM (fine quartz sand +20% kaolin), this would make it impossible to compare the toxicity of PVC-MP and natural particles as both would be present in a mixture. Additionally, increasing PVC-MP and constant quartz/kaolin concentrations would change the sediment characteristics by increasing the total amount of FPM. Therefore, we used sediments consisting of washed and sieved quartz sand (250–1000 μm), ground and sieved leaves of *Urtica dioica* and *Alnus glutinosa* (< 250 μm, 1:1 mixture based on mass) as carbon source and either PVC, kaolin or diatomite. Sediments for each replicate were individually prepared and mixed in 500 mL glass beakers.

2.1.1. Experiment I (exposure via sediment)

Each exposure vessel contained 100 g sediment (dry weight) consisting of sieved quartz sand, 1% leaves and 0.002, 0.02, 0.2, 2 and 20% (wt) of PVC, kaolin or diatomite. The sediments were homogenised and 400 mL Elendt M4 medium was carefully added per replicate to prevent suspension of particulate matter (n = 3 per treatment). Control treatments consisted of sediments without FPM.

2.1.2. Experiment II (exposure via water)

To evaluate the impact of a waterborne exposure, sediment compositions remained similar to experiment I except that the FPM were applied by suspending 0.0002, 0.002, 0.02, 0.2 and 2 g of PVC, kaolin or diatomite in 400 mL Elendt M4 medium (n = 3). FPM were weighed into 50 mL tubes per replicate, suspended in 50 mL Elendt M4 medium and shaken for 24 h in the dark (300 rpm, orbital shaker). One day later, the suspensions were transferred to the exposure vessels and the tubes were rinsed three times with fresh medium. The FPM were allowed to settle for 5 d before the toxicity experiment was started.

2.1.3. Experiment III (leaching of toxicity)

To investigate the toxicity of chemicals leaching from PVC-MP, we exposed chironomids to MP extracts (in solvent) and migrates (in medium). PVC-MP extracts were obtained by a 24 h Soxhlet extraction of 200 g PVC-MP using acetone. The extracts' volume was reduced to 50 mL using a rotary evaporator (Heidolph, Laborota 4000) and used to prepare diluted extracts equivalent to 0.2 and 20 g PVC-MP/10 mL acetone. An empty Soxhlet cartridge was extracted as blank. The sediments contained sieved quartz sand, 1% leaves and the corresponding amount of kaolin as FPM (0, 0.2 and 20%) and were spiked with 10 mL extracts, thoroughly mixed, followed by a 24 h period allowing the acetone to evaporate completely (n = 4). PVC-MP migrates were prepared by shaking a suspension of 20 g PVC-MP in 400 mL Elendt M4 medium for 33 d under the same conditions used for the toxicity experiments. The suspensions were filter using a glass fibre filter (1.5 μm pore size) and the filtrate was used as test medium. As a control, identically treated Elendt M4 medium without PVC-MP was used. The sediments contained sieved quartz sand, 1% leaves and 20% kaolin as FPM to have a similar size composition as in the other sediments (n = 4).

2.1.4. Experiment IV (pesticide co-exposure)

Chironomids were exposed to 0, 0.5, 1, 2, 4 and 8 $\mu g\ L^{-1}$ imidacloprid (nominal concentration) and sediments containing sieved quartz sand, 1% leaves and 0, 0.2 and 20% kaolin or PVC-MP as FPM (n = 4). We excluded diatomite to ensure the feasibility of parallel testing. Water and sediment from an extra replicate per treatment was sampled after 5 d for chemical analysis.

2.2. Experimental set up

All beakers were covered with nylon meshes and constantly aerated to provide sufficient oxygen. After a 5-d incubation period at 20 °C and a light:dark cycle of 16:8 h, aeration was stopped and 20 *C. riparius* larvae (< 24 h) were added per replicate (day 0). On the following day, the aeration was continued, and the larvae were fed with 0.2 mg fish food larva^{-1} d^{-1} (ground TetraMin suspended in Elendt M4 medium) during the first 7 d and 0.5 mg food larva^{-1}d^{-1} afterwards. During 28 d, the number of emerged imagoes was recorded daily. Emerged imagoes were counted, removed and snap-frozen for further analysis. To measure their weight, frozen specimens were defrosted and dried at 60 °C for 24 h. The dried individuals were weighted and sexed. Water quality parameters (oxygen level, pH and conductivity) were measured in 20 randomly selected vessels at the beginning and the end of the experiments. Here, validity of the experiments was given when the oxygen levels were > 85% and the pH was constant at 8.20 ± 0.35 in any vessel.

2.2.1. PVC-MP, phthalate and imidacloprid analysis

PVC-MP were analysed using thermal desorption gas chromatography coupled to mass spectrometry (TD-GC–MS, Multi-Shot Pyrolyzer EGA/PY-3030D coupled to an Agilent 7890B gas chromatograph and Agilent 5977B MSD, analytical details in Table S1). Imidacloprid, phthalates (dimethyl phthalate, diethyl phthalate, dibutyl phthalate, benzyl butyl phthalate, bis(2-ethylhexyl) phthalate, di-n-octyl phthalate, diisononyl phthalate) and bis(2-ethylhexyl) adipate were quantified in water or sediments using liquid chromatography coupled to mass spectrometry (LC-MS, Agilent 1260 coupled to a QTrap 4500 AB Sciex, analytical details in Table S1). 10 mL water and 30 g sediment were sampled from (a) analytical replicates at day 0 and (b) one randomly selected replicate per treatment at day 28 of experiment IV. These samples were snap frozen and stored at −20 °C until further processing (see SI for details).

2.3. Statistical analysis

Emergence, mean emergence time (EmT$_{50}$), mean development rate and the weight of male and females were used to evaluate the toxicity of PVC-MP, kaolin, diatomite and imidacloprid.

Mean development rates ($\bar{x}$; d^{-1}) were calculated for each replicate of every treatment in every experiment as:

$$\bar{x} = \sum_{i=1}^{m} \frac{f_i \times x_i}{n_e} \tag{1}$$

with m = the number of inspections, f_i = number of emerged individuals since the last inspection, x_i = time of inspection (d^{-1}), and n_e = total number of emerged individuals at the termination of the experiment. x_i was calculated as:

$$x_i = \left(\text{day}_i - \frac{l_i}{2}\right)^{-1} \tag{2}$$

with day$_i$ = day of inspection since the beginning of the experiment (d) and l_i = time interval since the last inspection (d) (OECD, 2004).

All data were analysed and visualised using R (version 3.5.1; R Core Team, 2018). Generalized linear models (GLMs) were fit for every endpoint (emergence, weight, EmT$_{50}$, and development rate) of each of the four experiments. For emergence and EmT$_{50}$, the distribution family was binomial with a logit link, as the data was expressed as either success or non-success (1 or 0, respectively). For weight and development rate, an exponential Gamma family distribution with inverse link was used because the variance in data increased with increasing average values.

All GLMs were initially fit with full parametrization, that is, with all experimentally possible effect interactions. To avoid over-fitting, all individual model parametrizations were stepwise optimized for minimum values of Akaike's information criterion (AIC; Akaike, 1974). A detailed list of all initial and final parametrizations of all GLMs is given in the supporting information (Table S2–8, Figure S4–10). For each GLM, the remaining parameters were analysed for effect strength in independent single-step term deletions with subsequent analysis of variance, using χ^2 tests with marginal (type III) sum-of-squares. An effect was considered significant for $p < 0.05$. Pseudo-R^2 values were calculated using McFadden's method (McFadden, 1979). Raw data and R scripts for data treatment, statistical analysis and visualisation can be made available upon request.

3. Results

3.1. Exposures to kaolin, diatomite and PVC-MP

3.1.1. FPM characterization and chemical analysis

On a microscopic scale, diatomite had a heterogeneous particle shape including sharp and needle-like fragments (Fig. 1). Kaolin and PVC-MP were more uniform and rounded. In suspension, all materials formed agglomerates. The size distributions (2–60 μm) confirm that the majority of FPM is < 16 μm (Fig. 1G–I). The mean sizes as well as the particle size distributions of kaolin (mean 2.80 μm with 90% of particles < 4.38 μm) and PVC-MP (mean 2.77 μm with 90% of particles < 4.25 μm) are similar. In contrast, diatomite particles are slightly larger (mean 4.44 μm with 90% of particles < 8.19 μm) and the size distribution lacks the exponential increase of smaller particles. According to the density of the materials, we expected the highest particle concentration for PVC-MP (1.4 g cm^{-3}), followed by diatomite (2.3 g cm^{-3}) and kaolin (2.6 g cm^{-3}). We experimentally determined the particle concentrations (numbers) per mass of 2–60 μm particles and observed a different order with kaolin (1.28×10^{10} particles g^{-1}) containing more particles per mass than PVC-MP (8.40×10^{9} particles g^{-1})

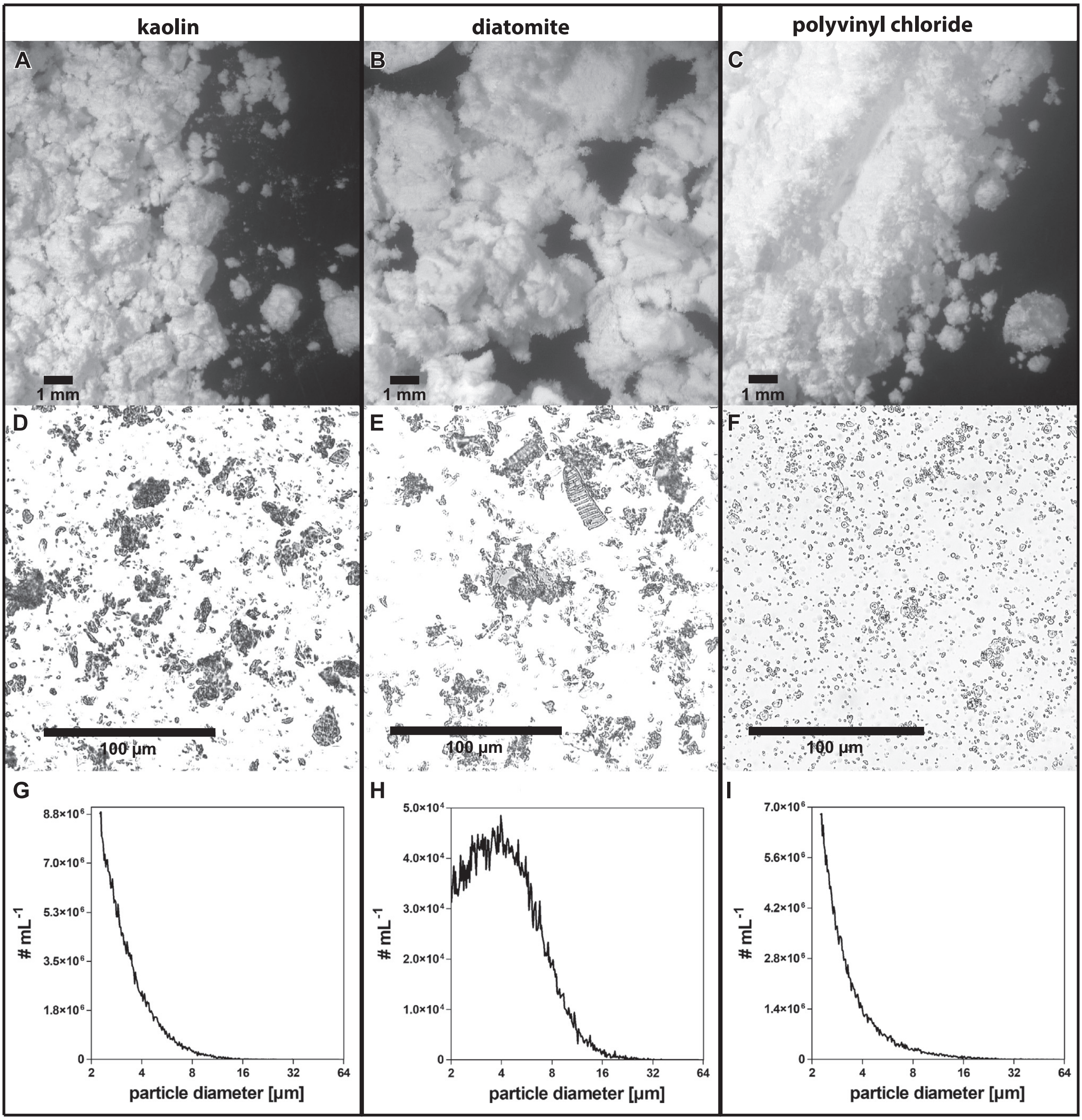

Fig. 1. Macro- and microscopic images (A-F) and size distributions (G-I) of kaolin (A, D, G), diatomite (B, E, H) and polyvinyl chloride microplastics (C, F, I).

and diatomite (5.25×10^9 particles g^{-1}, Figure S1A). We used these particle concentrations to calculate their mass based on the density and assuming spherical particles (Figure S1B). Indeed, predicting the mass of heterogeneously shaped and porous fragments is biased. However, the discrepancy in estimated and observed mass demonstrates that particles in the size range of 2 to 60 μm do not represent the total number and mass of particles and point to a missing fraction below the limit of quantification (0.68 μm) for kaolin (recovery of 85.2 ± 6.7%) and PVC-MP (recovery of 55.4 ± 11 .6%). This is supported by the exponentially increasing concentrations of particles < 2 μm for PVC-MP and kaolin (Figure S1C). Here, filtered (1.5 μm pore size) suspension of 50 g L^{-1} kaolin, diatomite and PVC-MP contain 1.59×10^4, 3.92×10^3 and 4.04×10^6 particles mL^{-1} in the size range of 0.68–2 μm, respectively.

According to the results of the thermal desorption GC–MS, PVC-MP does not contain additives. In addition, the measured concentrations of phthalates in the water and sediment samples are in the same range as blanks and controls, even at the highest PVC-MP concentration (Figure S2). The electrical conductivity differs between the 20% PVC-MP treatment (1347 ± 31 μS cm^{-1}) and all other treatments (1015 ± 44 μS cm^{-1}).

3.1.2. Effect of FPM on emergence and weight of *Chironomus riparius*

3.1.2.1. Exposures via sediment. In contrast to kaolin ($p > 0.05$), PVC-MP ($\chi^2 = 27.9$, $p < 0.001$) and diatomite ($\chi^2 = 4.54$, $p < 0.01$) significantly reduce the emergence of chironomids (Fig. 2A, Table 1, S2). FPM exposure significantly affects the mean time of emergence with material-specific outcomes (Table 1, Table S2, S6). For instance, increasing kaolin concentrations induce an earlier emergence of both sexes (Table S6, males: −0.47 d, females: −0.32 d). High concentrations of diatomite prolong the period between the emergence of males (−0.23 d) and females (+0.18 d) compared to sand only exposures. PVC-MP delay male emergence by 0.96 d whereas females emerge 0.27 d earlier (Table S6).

The weight of emerged imagoes depends on the sex with females being heavier than males (χ^2 = 9170, $p < 0.001$, Fig. 2B). Exposures to kaolin (χ^2 = 4.3, $p < 0.05$), diatomite (χ^2 = 31.2, $p < 0.001$) and PVC-MP (χ^2 = 46.1, $p < 0.001$) significantly affect the weight of male and female chironomids (Table 2). Here, increasing concentrations reduce the weight of male and female chironomids by 1.14% and 10.6% for diatomite and 0.23% and 16.7% for PVC-MP, respectively, compared to the treatments without FPM (Fig. 2B). Although the impacts on weight do not follow a clear dose–response pattern, the GLM suggests a slight decrease in weight with increasing kaolin concentrations (χ^2 = 4.3, $p < 0.05$, Fig. 2B, Table S6).

In summary, the impacts of FPM are minor at concentrations < 2% and become more pronounced at the highest concentration. At 20% FPM, the emergence of chironomids exposed to PVC-MP or diatomite is reduced by 32.7% and 12.7%, respectively, and generally delayed for PVC-MP (male: 1.43 d, female: 0.05 d) and diatomite (male: 0.24 d, female: 0.5 d), compared to the corresponding kaolin exposure. In addition, the weight of chironomids exposed to diatomite (male: −3.36%, female: −9.56%) and PVC-MP (male: −2.48%, female: −15.7%) is lower than in the kaolin treatments.

3.1.2.2. Exposure via water. The emergence is not affected by any FPM when *C. riparius* is exposed via water (Fig. 2C, Table 1, S3). In contrast, PVC-MP (χ^2 = 35.1, $p < 0.001$) and diatomite (χ^2 = 9.6, $p < 0.05$) significantly affect the emergence time with an earlier emergence of chironomids exposed to 2% PVC-MP compared to kaolin (male: −0.35 d, female: −0.55 d, Table S6) and diatomite (male: −0.23 d, female: −0.73 d, Table S6). The weight of emerged chironomids is significantly affected by kaolin (χ^2 = 5.3, $p < 0.05$) and PVC-MP (χ^2 = 45.2, $p < 0.001$, Fig. 2D, Table 1). Here, exposures to 2% kaolin (male: 0.79 ± 0.07 mg, female: 1.86 ± 0.19 mg) and 2% PVC-MP (male: 0.7 ± 0.06 mg, female: 1.6 ± 0.14 mg) increase and reduce the mass of emerged chironomids, respectively, compared to the treatments without FPM (male: 0.74 ± 0.15 mg, female: 1.79 ± 0.14 mg). However, overlapping confidence intervals of EmT_{50} (Table S6), high inter-replicate variability (20–100% emergence, Fig. 2C) and a generally reduced emergence (75.8 ± 16.3%, Fig. 2C) limit the conclusions that can be drawn from these observations.

3.1.2.3. Exposures to PVC-MP migrates and extracts. Exposures to PVC-MP migrates reduce the emergence success by 55.4% (Fig. 2E) and prolong the emergence time (male: 0.99 d, female: 1.33 d) compared to the control (M4 filtrate). The emergence is also affected in chironomids exposed to PVC-MP extracts equivalent to 0.2 (−10.1%) and 20% (−18.8%), whereas EmT_{50} values are decreased by 0.001–0.018 d (0.2%) and 0.06–0.8 d (20%) compared to the solvent control (Table S7). PVC-MP migrates and extracts do not affect the weight of chironomids (Fig. 2F).

3.2. Co-exposure to imidacloprid and kaolin or PVC-MP

3.2.1. Chemical analysis

The recovery rate of imidacloprid is 94.7 ± 12.7% at day 0 and 87.4 ± 14% at day 28 (Figure S3). Thus, nominal concentrations

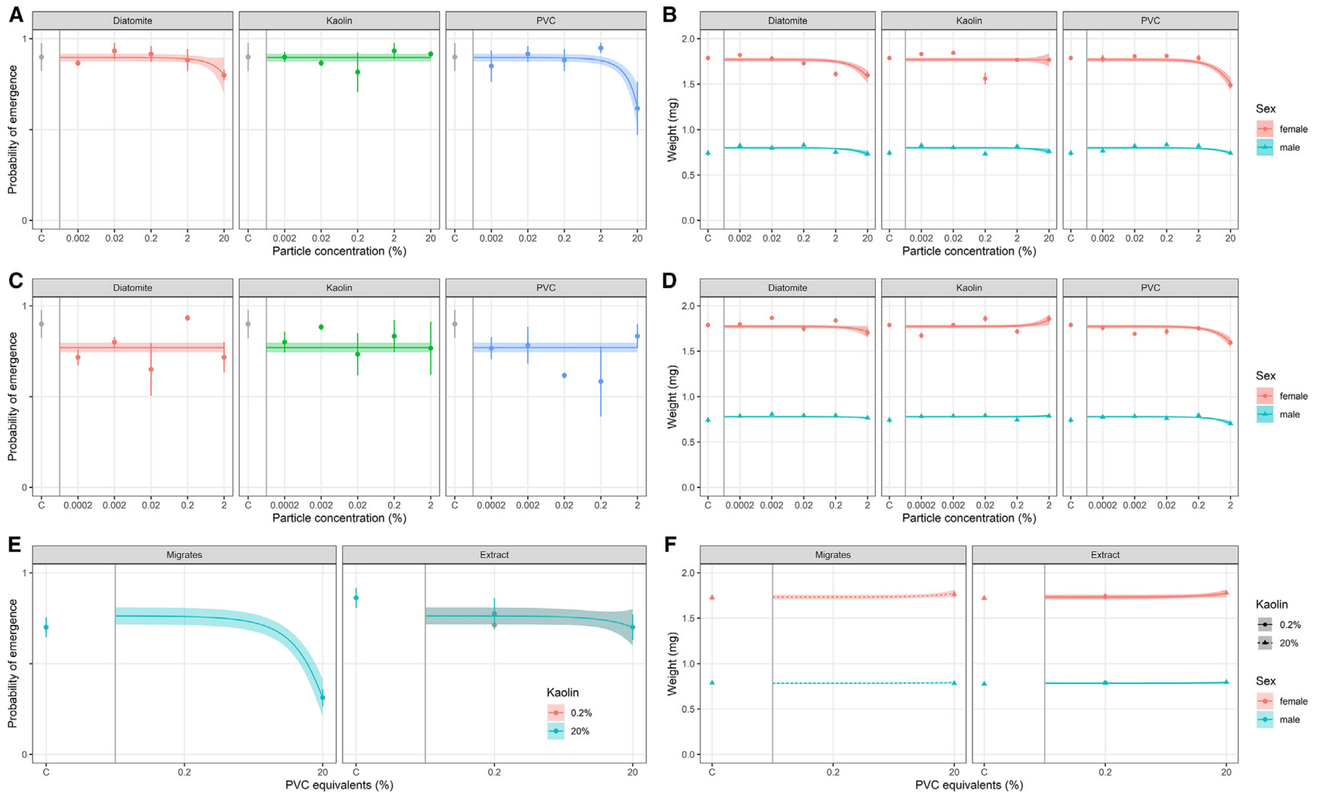

Fig. 2. Effects of kaolin, diatomite and PVC-MP as well as of migrates and extracts of PVC-MP on the emergence (A, C, E) and weight (B, D, F) of *Chironomus riparius* exposed via sediment (A, B) and water (C, D) or to PVC-MP migrates/extracts (E, F). Points are mean ± standard error of the mean. Regression lines show the results from individual generalized linear models (GLMs). For A, C, and E, GLMs were based on a binomial distribution with logit-link, whereas for B, D, and F, the GLMs were based on a Gamma distribution with inverse link. Areas around the regression lines represent the 95% confidence bands.

Table 1
Overview of the statistical analysis of single coefficients and interactions derived by generalized linear models.

				Exposure				
		Pseudo-R^2	SEX	PVC	KAO	DIA	IMIDA	Significant interaction(s)
Sediment	Emerg.	0.04	–	$p < 0.001$	–	$p < 0.05$	NA	–
	EmT$_{50}$	0.71	–	$p < 0.001$	$p < 0.001$	$p < 0.001$	NA	Sex:PVC ($p < 0.001$),Sex:Kaolin, Sex:Diatomite ($p < 0.01$)
	DR	0	–	–	–	–	NA	–
	Weight	0.92	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	NA	Kaolin:Sex ($p < 0.05$)
Water	Emerg.	0	–	–	–	–	NA	–
	EmT$_{50}$	0.76	$p < 0.01$	$p < 0.001$	–	$p < 0.01$	NA	Sex:Kaolin, Sex:PVC, Sex:Diatomite ($p < 0.01$)
	DR	0.01	–	–	–	–	NA	–
	Weight	0.95	$p < 0.001$	$p < 0.001$	$p < 0.05$	–	NA	PVC:Sex ($p < 0.05$)
Migrates & extracts	Emerg.	0.09	–	$p < 0.001$	–	NA	NA	PVC:Method ($p < 0.001$)
	EmT$_{50}$	0.76	$p < 0.01$	$p < 0.001$	$p < 0.001$	NA	NA	PVC:Method ($p < 0.001$)
	DR	0.1	–	–	–	NA	NA	–
	Weight	0.96	$p < 0.001$	–	–	NA	NA	–
IMIDA+KAO or PVC	Emerg.	0.58	–	$p < 0.001$	$p < 0.01$	NA	$p < 0.001$	PVC:IMIDA ($p < 0.001$), Kaolin:IMIDA ($p < 0.01$)
	EmT$_{50}$	0.81	$p < 0.001$	$p < 0.001$	$p < 0.001$	NA	–	Kaolin:IMIDA ($p < 0.01$), PVC:IMIDA ($p < 0.05$)
	DR	0.65	–	$p < 0.001$	$p < 0.001$	NA	$p < 0.001$	–
	Weight	0.92	$p < 0.001$	$p < 0.001$	$p < 0.001$	NA	$p < 0.001$	PVC:IMIDA, PVC:Sex, Kaolin:Sex ($p < 0.001$)

Emerg. = emergence success, EmT$_{50}$ = mean emergence time, DR = development rate, KAO = kaolin, DIA = diatomite, IMIDA = co-exposure to imidacloprid. NA = not available. – = no significant difference. For exact *p* values and details on the GLMs see Supporting Information.

Table 2
EC$_{10}$ and EC$_{50}$ [mg L^{-1}] values for the emergence success of *Chironomus riparius* exposed to imidacloprid and kaolin or PVC.

	0%	0.2%		20%	
	Sand	Kaolin	PVC	Kaolin	PVC
EC$_{10}$	1.20	1.20	1.18	1.64	0.29
(95% CI)	(1.04–1.36)	(1.05–1.36)	(1.03–1.34)	(1.41–1.91)	NC
EC$_{50}$	2.64	2.64	2.63	2.75	1.52
(95% CI)	(2.50–2.79)	(2.50–2.79)	(2.49–2.78)	(2.54–2.99)	(1.22–1.87)

CI = confidence interval, NC = not calculable.

were used for data analysis. During 28 d, we observed only a minor change in the partitioning of imidacloprid: At day 0, 88.4 ± 4% is dissolved in the water phase and 11.6 ± 4% associated with the sediment. At the end of experiment IV, the imidacloprid concentration in water decreases to 80 ± 4% and increases to 20 ± 7% in the sediment. This observation was especially pronounced in treatments containing high FPM levels (20% kaolin/PVC, Figure S3B). A maximum partitioning of 27.5% of imidacloprid to sediments containing 20% FPM indicates that sorption is minor and chironomids are mainly exposed to dissolved imidacloprid.

3.2.2. Effect of PVC-MP and kaolin on C. Riparius

The GLMs (Fig. 3A) confirm the previous findings that kaolin exposure positively affects the emergence success (χ^2 = 13.3, $p < 0.01$) and time (χ^2 = 16.4, $p < 0.001$) while an exposure to 20% PVC-MP has the opposite effect (χ^2 = 205, $p < 0.001$, Table 1, S6). For instance, without imidacloprid, the number of emerged chironomids increases from 92.5 to 100% and the EmT$_{50}$ decreases with increasing kaolin concentration (males: −0.6 d, females: −0.64 d, Table S8). For PVC-MP, the number of emerged chironomids decreases from 92.5% to 47.5% and the EmT$_{50}$ increases with increasing concentration (males: +3.95 d, females: +4.55 d, Table S8). In addition, the weight of male and female chironomids is significantly reduced when exposed to 20% kaolin (males: −3.55%, females: −5.80%, χ^2 = 159, $p < 0.001$) and 20% PVC-MP (males: −23.5%, females: −21.6%, χ^2 = 213, $p < 0.001$, Table 1, Fig. 3). While chironomids from the 0.2% PVC-MP/kaolin treatments weigh approximately the same, an exposure to 20% PVC-MP decrease the weight by −15.0% (males) and −19.5% (females) compared to treatments containing 20% kaolin. In contrast to the previous experiments, kaolin (χ^2 = 12.7, $p < 0.001$) and PVC-MP (χ^2 = 120, $p < 0.001$) significantly affect the development rate (Table 1). Increasing kaolin concentrations increase the development rate, while chironomids exposed to PVC-MP develop slower with increasing concentrations (Figure S10). Thus, adverse impacts of 20% PVC-MP on the emergence success and time, development rate and weight of imagoes are more pronounced than in experiment I.

3.2.3. Effect of imidacloprid on C. Riparius

Imidacloprid significantly (χ^2 = 1119, $p < 0.001$) affects the emergence of chironomids in a concentration-dependent manner (Table 1, Fig. 3A). Regardless the FPM, an exposure to 4 and 8 µg L^{-1} imidacloprid results in 90 and 100% mortality, respectively. Although imidacloprid alone has no significant influence on the emergence time (Table 1), the EmT$_{50}$ slightly increases with an increasing concentration (0–2 µg L^{-1}: males: +1.1 d, females: +1.05 d, Table S8). In the treatment with 4 µg L^{-1} imidacloprid, the reduced emergence is accompanied by an additionally delayed development (males: +1.2 d, females: +1.61 d). In addition, the development rate (χ^2 = 14.8, $p < 0.001$) as well as the weight (χ^2 = 152, $p < 0.001$) of chironomids significantly decrease with increasing imidacloprid concentrations (Table 1, Figure S10).

3.2.4. Interactions of FPM and imidacloprid

There are significant interactions of FPM and imidacloprid for emergence success and time as well as weight in the GLM. This implies that co-exposure to FPM modulates the toxicity of imidacloprid. For instance, the significant interaction between kaolin: imidacloprid (χ^2 = 8.58, $p < 0.01$) and PVC:imidacloprid (χ^2 = 17.3, $p < 0.001$) on the emergence success is mirrored by the EC$_{50}$ values for emergence. The latter demonstrate that the joint toxicity depends on the material with kaolin being less toxic than PVC-MP (Table 2). Here, the influence of sand, 0.2% kaolin, 0.2% PVC-MP and 20% kaolin is minor (overlapping confidence intervals). In contrast, co-exposure to 20% PVC-MP markedly increases the imidacloprid toxicity (Fig. 3A).

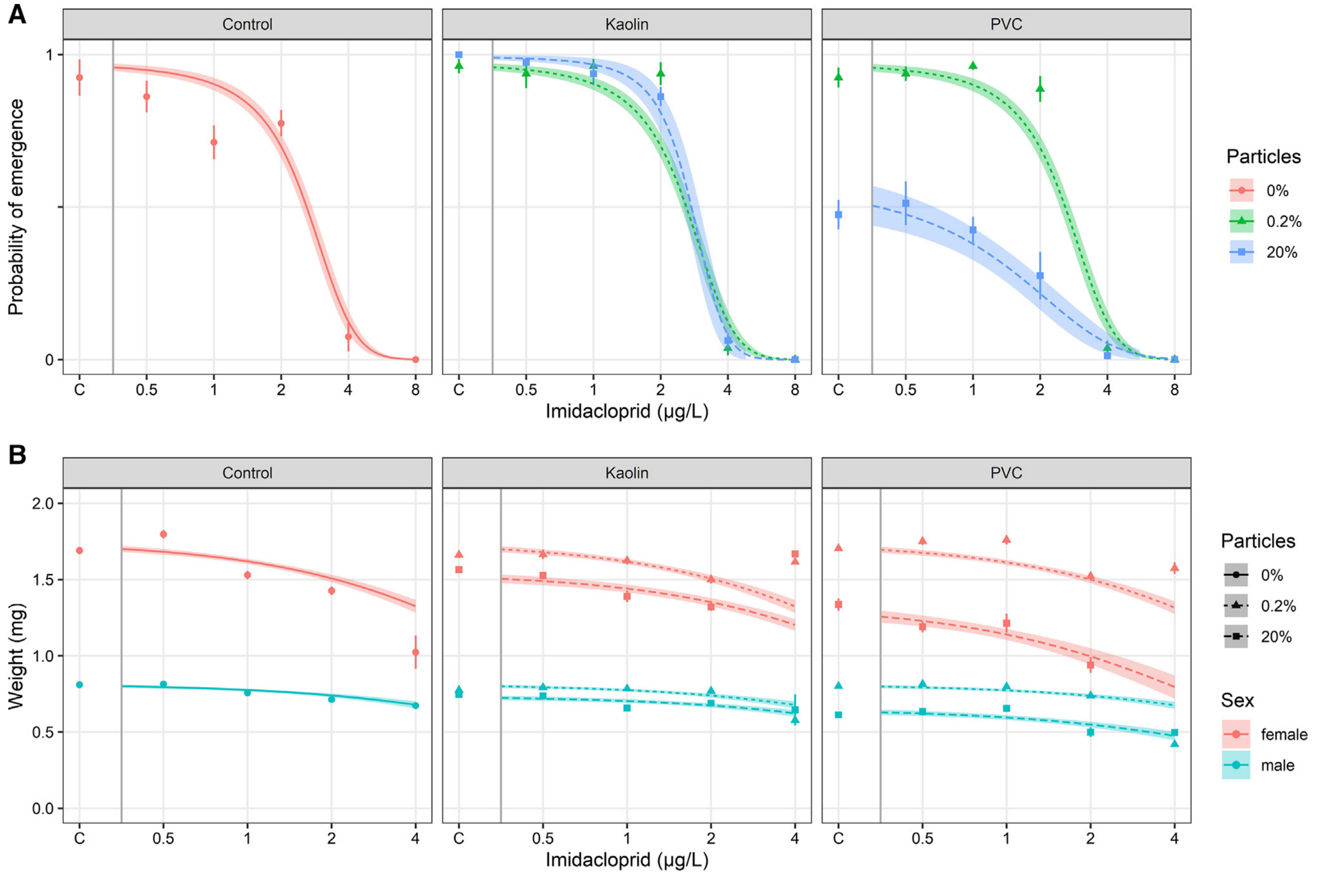

Fig. 3. Effects of a co-exposure to imidacloprid and kaolin or PVC-MP on the emergence (A) and weight (B) of *Chironomus riparius*. Points are mean ± standard error of the mean. Regression lines show the results from individual generalized linear models (GLMs). For A, the GLM was based on a binomial distribution with logit-link, whereas for B, the GLM was based on a Gamma distribution with inverse link. Areas around the regression lines represent the 95% confidence bands.

For the emergence time, the GLM shows significant interactions between kaolin:imidacloprid (χ^2 = 10.6, $p < 0.01$) and PVC:imidacloprid (χ^2 = 5.7, $p < 0.05$). The EmT_{50} values indicate that a higher proportion of kaolin reduces the delaying effect on the emergence induced by imidacloprid whereas a higher proportion of PVC-MP exacerbates this effect (Table S8). For instance, increasing the concentration of imidacloprid from 0 to 2 μg L^{-1} and the proportion of FPM from 0.2 to 20%, reduces the EmT_{50} by 0.56 d (males) for kaolin and increases the EmT_{50} by 0.53 d (males) for PVC-MP. In addition, the interaction between PVC-MP and imidacloprid significantly affect the weight of chironomids (χ^2 = 21.4, $p < 0.001$, Fig. 3B, Table 1). For instance, increasing the concentration of imidacloprid from 0 to 2 μg L^{-1} and the proportion of PVC-MP from 0.2 to 20%, results in a reduction of weight by 32.5% (males) and 38.2% (females).

4. Discussion

Chronic exposures to PVC-MP and natural particles significantly affect the emergence and weight of *Chironomus riparius*. These effects are material-specific with PVC-MP being more toxic than the natural kaolin and diatomite. In addition, kaolin slightly reduces while PVC-MP exacerbates the toxicity of imidacloprid by acting as an additional stressor. Given the high concentrations needed to induce adverse effects, *C. riparius* seems to tolerate PVC-MP exposures at levels much higher than currently expected in the environment.

Overall, it remains challenging to determine which particle and material properties drive the toxicity of FPM in chironomids. Here, we tried to match the multiple properties (Table 3) and show that PVC-MP and kaolin are very similar regarding their shape and size distribution. Accordingly, the higher toxicity of PVC-MP may be caused by the presence of very small particles, its surface chemistry or its chemical composition. In contrast, diatomite is generally larger and contains less particles < 2 μm than PVC-MP and kaolin, which leads to the assumption that the spiny and needle-like shape as well as the high porosity contribute to the toxicity.

Taken together, this study empirically supports previous calls to consider the heterogeneous properties of MP and other types of particulate matter when assessing their toxicity (Rochman et al., 2019; Hartmann et al., 2019; Lambert et al., 2017). A detailed discussion of our findings and potential mechanisms for FPM toxicities in chironomids are provided in the following section.

4.1. Exposure to PVC-MP and other FPM affects the emergence and weight of C. Riparius

4.1.1. The toxicity of kaolin, diatomite and PVC-MP is material-dependent

Exposure to diatomite and PVC-MP significantly reduces the emergence and weight of chironomids (Fig. 2). In contrast, kaolin positively affects the chironomids by increasing the emergence success and decreasing the emergence time. The beneficial effect of kaolin is in line with its application as sediment component in standardized toxicity testing (OECD, 2004) and highlights that chironomids depend on FPM present in sediments. Moreover, because its particle size distribution and shape are similar to PVC-MP, kao-

Table 3
Comparison of the properties of fine particulate materials used in this study.

		PVC	vs	kaolin	vs	diatomite	PVC vs diatomite
2–60 µm	mean size [µm]	2.77 [2.75-2.78]	=	2.80 [2.78-2.81]	<	4.44 [4.36-4.53]	<
	#P/20 g	1.68×10^{11}	<	2.55×10^{11}	>	1.05×10^{11}	>
0.68–2 µm	mean size [µm]	0.86 [0.85-0.86]	<	0.94 [0.90-0.98]	<	1.38 [1.34-1.43]	<
	#P/mL	4.04×10^{6}	>	1.59×10^{4}	>	3.92×10^{3}	>
	shape	rounded fragments	=	rounded fragments	≠	sharp/spiny fragments	≠
	surface	hydrophobic	≠	hydrophilic	=	hydrophilic	≠
	add. information	no organic additives		inert		inert, high porosity	

Measurements of mean size [µm] and particle concentration, classification of shape via microscopy, surface characteristics and additional information of kaolin (Murray, 2006) and diatomite (Hadjar et al., 2008) from the literature.

lin appears to be a suitable reference material for this type of MP. At the same time, diatomite may be a suitable counterpart when assessing particle related toxicities in chironomids because it represents different physical properties (sharpness and porosity).

PVC-MP is more toxic than natural particles. Adverse effects on the emergence and weight of chironomids become evident at the highest applied concentrations (20% FPM exposed via sediment and 2% exposed via water) with PVC-MP being more toxic than diatomite and kaolin (Fig. 2). The same is true when comparing the joint effects of particles in combination with imidacloprid (Fig. 3). Diatomite induced adverse effects (see above) which were lower than the ones of PVC-MP at the same mass-based concentration. However, when comparing numerical concentrations instead, diatomite was more toxic because it contains less particles per mass (Table 3).

4.1.2. The toxicity of kaolin, diatomite and PVC-MP is concentration-dependent

As indicated by the GLMs, FPM gradients significantly affect the probability and time of emergence as well as the weight of emerged chironomids in a material-specific manner (Table 1, Fig. 2). This is in accordance with literature on MPs and natural FPM reporting that the results of an exposure strongly depends on the dose and material (reviewed in Scherer et al., 2017b). In general, increasing FPM concentrations will increase the encounter rates (physical) and/or exposure concentrations (chemical) and, thus, increase material-related toxicities. Consequently, low FPM concentrations in mixed sediments (< 2%) correspond to low encounter rates and thus, minimize particle-related effects. Such dilution in the sediment does not occur for exposures via the water phase because, during the 5 d incubation period, the FPM settled on the sediment surface resulting in a top layer consisting almost exclusively of FPM at the highest concentration (2%). This might explain the significantly affected weight of imagoes in those treatments. Accordingly, the spiking method strongly influences the outcomes of toxicity studies with MPs.

4.1.3. A comparison with the literature points to species- and material-specific effects

Our results are in accordance with literature, as many studies on MPs do not observe adverse effects in freshwater invertebrates at low concentrations (Imhof et al., 2017; Imhof and Laforsch, 2016; Weber et al., 2018). In contrast, Ziajahromi et al. (2018) observed size-specific effects at much lower MP concentrations in *Chironomus tepperi*. Exposure to 500 PE-MP kg^{-1} sediment, reduced survival, emergence, body length and growth for 10–27 μm MP, while 100–126 μm PE-MP did not affect the survival but increase the emergence time. Indeed, species-specific sensitivities (*C. riparius* vs *C. tepperi*), material-specific toxicities (PVC vs PE) as well as the different experimental setup (28 vs 10 d) might provide an explanation. In addition, a recalculation of MP concentrations based on the given density (0.98–1.02 g cm^{-3}), size (1–126 μm) and mass (300–500 mg L^{-1}) of the PE-MP used by Ziajahromi et al. (2018) reveals a discrepancy. Theoretically, the exposure concentrations for PE-MP < 27 μm are 48 to 57,500 times higher than stated in the study. However, when comparing the effects of the two studies based on mass not particle concentrations, it becomes clear that *C. tepperi* is either more sensitive to MP exposure than *C. riparius* or that PE-MP is more toxic than PVC-MP. Here, exposure studies with *C. riparius* and PE-MP confirm both assumptions (Silva et al. 2019). However, exploring this interesting question would require identical experimental setups (exposure regimes and materials). Nevertheless, previous studies did not include natural reference particles or adapted sediment compositions to characterise MP-specific effects. In this regard, our results highlight that material-specific properties of PVC-MP induce a stronger toxicity than natural particles.

4.1.4. PVC-MP affects C. Riparius at concentrations much higher than currently detected in the environment

We observed a reduced and delayed emergence as well as a reduced weight of imagoes at high, environmentally irrelevant exposures. While reports on mass-based MP concentrations in freshwater sediments are scarce, concentrations between 2.6 (18 particles kg^{-1}) and 71.4 mg kg^{-1} (514 particles kg^{-1}) have been reported for riverine sediments (Rodrigues et al., 2018). The low numerical compared to the given mass concentrations indicate that mainly larger MPs have been analysed. In general, environmental levels of MPs < 63 μm remain largely unknown due to the methodological challenges of separation and identification (Burns and Boxall, 2018; Conkle et al., 2018). Nonetheless, sediment contamination with 20% MP is highly unlikely. However, we used such high concentrations of MP not to mimic environmental conditions but to better understand how different FPM affect chironomids. Overall, *C. riparius* tolerates exposures to high concentrations of FPM, including unplasticised PVC-MP.

4.2. Potential mechanisms of FPM toxicity

4.2.1. The availability of utilisable FPM affects chironomids

The autecology of chironomids is an important factor when assessing the toxicity of FPM. As rather non-selective deposit feeders, they actively ingest various particles including detritus, algae, silt and MPs in the size range of 1–90 μm (Nel et al., 2018; Rasmussen, 1984; Scherer et al., 2017a; Ziajahromi et al., 2018). They mainly interact with particles on sediment surfaces and are tolerant to various sediments as long as FPM for tube building and foraging are available (Brennan and McLachlan, 1979; Naylor and Rodrigues, 1995; Suedel and Rodgers, 1994). Since we removed the fine fraction from the sediments, only the ground leaves and the FPM are in the utilizable size range. At low FPM-concentrations, larvae utilized grounded leaf fragments for tube building and with increasing concentrations mostly FPM. Thus, modifying the sediment compositions is an appropriate tool to highlight the material-specific toxicities in chironomids.

The overall beneficial effect of kaolin on the emergence of chironomids is most likely related to the increased availability of utilizable particles. In theory, these reduce the time and energetic effort of larvae to build and maintain stable tubes and, thus, increase the time available for foraging on the sediment surface (Naylor and Rodrigues, 1995). Our findings support this assumption as chironomids develop faster and obtain higher emergence rates when exposed to 20% kaolin (experiment I and IV). In contrast, 20% kaolin reduces the weight of imagoes (experiment IV) probably by decreasing the dietary uptake by the larvae (Ristola et al., 1999; Sibley et al., 2001). Both findings highlight that natural FPM can be beneficial for some life history parameters of chironomids by providing tube-building materials as well as negative for others by reducing food availability.

4.2.2. Diatomite is a suitable reference material for shape-specific effects

The adverse effects of diatomite on chironomids are not surprising as it is used as a natural pesticide in agriculture (Kavallieratos et al., 2018; Korunic, 1998). The spiny and porous fragments are supposed to damage the digestive tract and disrupt the functionality of the cuticle by sorption and abrasion (Korunic, 1998). Therefore, the observed effect of diatomite seems plausible and, in addition, is in accordance with literature (Bisthoven et al., 1998). While this mode of action is plausible for diatomite, kaolin and PVC-MP have rounder shapes (Fig. 1, Table 3). Thus, other properties than particle shape drive the toxicity of PVC-MPs in chironomids.

4.2.3. Leaching chemicals are of minor importance for the toxicity of unplasticised PVC-MP in chironomids

The leaching of chemicals is a commonly addressed issue in toxicity studies with PVC (Lithner et al., 2011, 2012b). We used unplasticised PVC on purpose to minimize the complexity of the experiments and confirm that the PVC-MP does not contain additives commonly used in plasticized PVC. Along that line, PVC extracts simulating a worst-case leaching induced limited toxicity in *C. riparius* (Fig. 2E–F). However, volatile or inorganic compounds (e.g., vinyl chloride monomer, Cd, Zn, Pb) may migrate from PVC to water (Ando and Sayato, 1984; Benfenati et al., 1991; Lithner et al., 2012a; Whelton and Nguyen, 2013). Due to constant aeration and the 5 d acclimatisation period, concentrations of volatile substances in our experiments are expected to be low. Nonetheless, chironomids are sensitive to heavy metals and migrated metal ions might contribute to the observed effects (Béchard et al., 2008; Timmermans et al., 1992). The slight increase in electric conductivity of treatments with either PVC-MP-migrates or sediments with 20% PVC-MP indicates a higher concentration of ions compared to the other treatments. As *C. riparius* is tolerant to high salinities, a general effect of high ion concentrations is unlikely (Bervoets et al., 1996). Accordingly, leaching chemicals did not contribute significantly to the toxicity observed for PVC-MP in our experimental setup.

However, the surface chemistry of PVC-MP might have induced toxicities in chironomids. In contrast to the hydrophilic silicate minerals kaolin ($Al_2H_4O_9Si_2$) and diatomite (mainly SiO_2), the surface of PVC-MP consists mainly of C—C, C—H and C—Cl moieties rendering it hydrophobic (Bakr, 2010; Murray, 2006; Asadinezhad et al., 2012). As surface charges and polarities are known to influence particle–particle and particle-biota interactions (e.g. aggregation, wettability), an effect induced by the hydrophobicity of PVC-MP on *C. riparius* seems likely (Nel et al., 2009; Potthoff et al., 2017).

4.2.4. Particles in the nanometre size range might have affected the toxicity of PVC-MP

In contrast to leachates, PVC-MP migrates produced under much milder conditions, significantly affected the emergence of chironomids. Since the concentrations of chemicals will be much lower than in the extracts, we speculate that particles in the nanometre size-range might have caused the observed effects (Table 1, Figure S1). Notwithstanding the similarities in shape and mean size, PVC-MP suspensions contain higher numbers of particles < 2 μm than kaolin (Table 3). Accordingly, this very small size fraction, including nanoplastics, present in the PVC-MP migrates might have induced the strong toxicity. While these particles would be also present in the MP suspensions used in the other experiments, the lower toxicity observed there may be due to a reduced bioavailability of nanoplastics attached to larger MP. The migrate toxicity highlights that the relevance of the smaller size fraction of particles present when using polydisperse MP mixtures. While it is technically challenging to remove nanoplastics from those, a comprehensive characterization will facilitate the interpretation of results.

4.2.5. PVC-MP >2 μm affects the weight of chironomids

In contrast to suspended PVC-MP, extracts and migrates significantly affected the emergence but not the weight of chironomids ($p < 0.001$, Table 1). Thus, the weight of imagoes is affected only if PVC-MP > 2 μm are present. Here, hydrophobic PVC-MP might have affected the weight by interfering with tube building and maintenance or with locomotion or digestion (Silva et al. 2019). A reduced feeding by food dilution in the sediment or the digestive tracts appears plausible, especially at high particle concentrations (Rist et al., 2016; Scherer et al., 2017b; Ziajahromi et al., 2018). However, the same mechanisms would be true for kaolin which did not reduce the body weight in the same way as PVC-MP (Figs. 2, 3). Accordingly, PVC-MP must have a more specific mechanism of action (e.g., hydrophobicity, chemical composition) affecting growth apart from a general physical toxicity.

4.3. Co-exposures to kaolin and PVC-MP affect the toxicity of imidacloprid in C. Riparius

4.3.1. Imidacloprid affects chironomids in a concentration-dependent manner

Imidacloprid induced adverse effects in chironomids in accordance with literature (Azevedo-Pereira et al., 2011; Langer-Jaesrich et al., 2010; Pestana et al., 2009). The neonicotinoid reduced the survival, emergence and growth of *C. riparius* in effect concentrations similar to our study. In addition, Pestana et al. (2009) and Azevedo-Pereira et al. (2011) observed significantly affected ventilation and locomotion behaviours. These effects are related to the mode of action of imidacloprid and an agonist of the postsynaptic nicotinic acetylcholine receptors disrupting neurotransmission (Matsuda et al., 2001; Tomizawa and Casida, 2005, 2003). Therefore, the reduced growth and affected emergence are mostly linked to an impaired foraging (Alexander et al., 2007; Roessink et al., 2013).

4.3.2. FPM modulate the toxicity of imidacloprid with material-specific outcomes

High concentrations of PVC-MP significantly exacerbated the adverse impacts of the neonicotinoid while kaolin has a slight compensatory effect (Tables 1 and 2, Fig. 3). Such modulation of toxicities by FPM is in accordance with literature. Here, elevated or reduced toxicities of chemicals are commonly associated with changes in bioavailability (Avio et al., 2015; Beckingham and Ghosh, 2017; Fleming et al., 1998; Landrum and Faust, 1994; Ma et al., 2016; Rochman et al., 2013). However, because we used a hydrophilic chemical not sorbing in relevant amounts to FPM, a modulation of imidacloprid bioavailability plays a minor role in the combined exposures.

PVC-MP and imidacloprid both affect the emergence and weight of chironomids (Fig. 3, Table 2). Here, co-exposure to 20% PVC-MP results in a 1.7-fold reduction of the EC_{50} of the neonicotinoid (Table 2). Considering the hypothesis that 20% PVC-MP directly (digestion) or indirectly (locomotion and tube building) interfere with foraging and feeding (4.2), a higher toxicity of a neurotoxic insecticide in the presence of an additional, particulate stressor is not surprising. In contrast, kaolin significantly interacts with imidacloprid by increasing the probability of emergence (Table 1, Fig. 3). As imidacloprid remains primarily dissolved in the water (> 72.4%) independent of the presence of FPM, any alterations in toxicity of imidacloprid are linked to an additional effect of the FPM. The slightly decreasing concentrations in the water and increasing concentrations of imidacloprid in the sediment over time are in accordance with the literature (Capri et al., 2001). Degradation (e.g., photolysis) might explain the slightly reduced imidacloprid concentration at day 28 (Figure S2B) whereas the high concentration of FPM probably affect the sorption capacity of sediments by increasing the reactive surface and potential binding sites (Capri et al., 2001; Cox et al., 1998). However, due to the high water solubility (610 mg L^{-1}) and low log K_{OW} (0.57), imidacloprid is expected to have a low affinity to bind on particulate matter and to accumulate in sediments (EC, 2006). In general, the capacity of PVC-MP to adsorb chemicals is considered to be low compared to other polymers (e.g., polyethylene) as the high crystallinity of PVC affects the diffusivity and, thus, the sorption of chemicals (Bakir et al., 2016, 2012; O'Connor et al., 2016; Teuten et al., 2009, 2007). Thus, our findings highlight that a co-exposure of MP and chemicals induces a stronger toxicity that is not caused by changes in bioavailability of the chemical stressor. Here, the beneficial effect of kaolin mitigates the toxicity of imidacloprid whereas PVC-MP enhances the toxicity at sub-lethal concentrations by acting as an additional stressor (Table 2).

5. Conclusion

This study shows that PVC-MP and natural particulate matter affects the emergence, development and weight of *Chironomus riparius* in a concentration-dependent manner. Kaolin and diatomite as natural reference for MP induced beneficial and negative effects, respectively. This demonstrates that natural FPM are not benign *per se* and that their impacts strongly depend on their physico-chemical properties, such as shape. PVC-MP induced more severe effects than both natural FPM highlighting that synthetic particles are more toxic than natural particles. As this was unrelated to leaching chemicals, other parameters, such as the presence of very small plastic particles or hydrophobicity may drive the toxicity of PVC-MP. Importantly, PVC-MP was toxic in high concentrations currently not detected in the aquatic environment. Same is true for co-exposures to the neonicotinoid imidacloprid. In a multiple-stressor experiment, high PVC-MP and kaolin concentrations increased and decreased the toxicity of imidacloprid in chironomids, respectively. Since imidacloprid is hydrophilic, other factors than bioavailability contributed to the combined effect of FPM and a chemical stressor. Although we observed a higher toxicity of PVC-MPs compared to natural particles, our results indicate that chironomids are very tolerant to an exposure to unplasticised PVC particles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contributions

C.S. designed the experiments and conducted the experimental work. J.V. produced the PVC-MP extracts and assisted in the experiments. C.S. and R.W. analysed the data and prepared the figures. C. S. and M.W. wrote the manuscript. N.B., F.S. and G.R. provided their feedback on the manuscript and guided the overall project. All authors reviewed the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134604.

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