



# Are bioplastics and plant-based materials safer than conventional plastics? *In vitro* toxicity and chemical composition



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## ABSTRACT

Plastics contain a complex mixture of known and unknown chemicals; some of which can be toxic. Bioplastics and plant-based materials are marketed as sustainable alternative to conventional plastics. However, little is known with regard to the chemicals they contain and the safety of these compounds. Thus, we extracted 43 everyday bio-based and/or biodegradable products as well as their precursors, covering mostly food contact materials made of nine material types, and characterized these extracts using *in vitro* bioassays and non-target high-resolution mass spectrometry. Two-third (67%) of the samples induced baseline toxicity, 42% oxidative stress, 23% antiandrogenicity and one sample estrogenicity. In total, we detected 41,395 chemical features with 186–20,965 features present in the individual samples. 80% of the extracts contained > 1000 features, most of them unique to one sample. We tentatively identified 343 priority compounds including monomers, oligomers, plastic additives, lubricants and non-intentionally added substances. Extracts from cellulose- and starch-based materials generally triggered a strong *in vitro* toxicity and contained most chemical features. The toxicological and chemical signatures of polyethylene (Bio-PE), polyethylene terephthalate (Bio-PET), polybutylene adipate terephthalate (PBAT), polybutylene succinate (PBS), polylactic acid (PLA), polyhydroxyalkanoates (PHA) and bamboo-based materials varied with the respective product rather than the material. Toxicity was less prevalent and potent in raw materials than in final products. A comparison with conventional plastics indicates that bioplastics and plant-based materials are similarly toxic. This highlights the need to focus more on aspects of chemical safety when designing truly “better” plastic alternatives.

## 1. Introduction

Bioplastics are promoted as an alternative to conventional petroleum-based non-biodegradable plastics. With a production volume of 2.11 million tons in 2018, their market share is very low (1% of all plastics) but expected to increase in the future (European Bioplastics, 2018). The term “bioplastics” is still ill defined. It includes materials made from renewable feedstocks (bio-based, e.g., Bio-polyethylene, Bio-PE), materials supposed to degrade naturally (biodegradable, e.g., polybutylene succinate, PBS), or both (e.g., polylactic acid, PLA; Lambert and Wagner, 2017). Similar materials on the market, such as starch blends, are also defined as bioplastics by European Bioplastics (2018). It is currently unclear whether those and other plant-based

materials that are often blends with synthetic materials (e.g., cellulose and bamboo-based materials) fall under that category. Either way, they are produced to fulfill the same function as plastic materials and appear as such to the consumer.

The term “bioplastics” implies that they have similar favorable characteristics as their petroleum-based counterparts (e.g., cheap, lightweight, flexible) but with the positive connotation of “natural” materials. Along that line, they are marketed as more sustainable and benign than conventional plastics. However, little scientific evidence supporting such notion exists. As an example, some biodegradable plastics do not degrade in industrial or natural settings (Haider et al., 2019). When evaluating and improving the environmental performance of bioplastics and plastic alternatives, the main focus is put either on

**Abbreviations:** Bio-PE, bio-based polyethylene; Bio-PET, bio-based polyethylene terephthalate; EC, effect concentration; hAR, human androgen receptor; hER $\alpha$ , human estrogen receptor  $\alpha$ ; IR, induction ratio; LOD, limit of detection; PBAT, polybutylene adipate terephthalate; PBS, polybutylene succinate; PHA, polyhydroxyalkanoate; PLA, polylactic acid; SD, standard deviation; UPLC-QTOF-MS/MS, ultra-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry; YES, Yeast Estrogen Screen; YAAS, Yeast Antiandrogen Screen

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the production stage (e.g., carbon footprint, renewable feedstocks) or at the end of life (e.g., degradability). Currently, the performance during the use phase, such as the human exposure to chemicals are often disregarded when evaluating the materials' sustainability (Ernstoff et al., 2019; Muncke et al. 2020). Along that line, very little is known in terms of the chemical safety of bioplastics, that is the identity of compounds present in the material and their (mixture) toxicity as well as the human exposure to these compound. These gaps in our knowledge are problematic because human exposure to chemicals from bioplastics and plant-based materials will increase with their increasing application.

Compounds intentionally used in plastics include additives such as plasticizers, antioxidants and stabilizers that improve the material's functionality as well as solvents and catalysts that enable production (Hahladakis et al., 2018). In addition, other intentionally (e.g., unreacted monomers) and non-intentionally added substances (NIAS, side or breakdown products) are present (Muncke, 2009). Although the individual compounds will be specific to the material, conventional as well as bio-based and biodegradable plastics can contain all these chemical categories. Additives are particularly relevant for polymers extracted from natural resources, such as starch and cellulose, or from microorganisms, such as PLA, because of their limited physical properties, such as thermal resistance and barrier properties (Beach et al., 2013; Khan et al., 2017). As most of these compounds are not covalently bound to the polymer, they can be transferred to air, solids (e.g., packed good or soil) or liquids (e.g., beverages) in a process called chemical migration. Thus, plastics are a major source of chemical exposures to humans (Muncke et al., 2020) and potentially also terrestrial and aquatic ecosystems.

In our previous study, we demonstrated that the majority of consumer products made of conventional plastics contains chemicals that are toxic *in vitro* (Zimmermann et al., 2019). Interestingly, this was also true for the small set of bioplastics we analyzed. Accordingly, the aim of this study was to investigate whether a broader set of bioplastics and plant-based materials contain chemicals inducing toxicity. We hypothesized that the *in vitro* toxicity of chemicals in bioplastics and plant-based materials is comparable to that of petroleum-based, non-biodegradable plastics and that the toxicity is more pronounced in the finished products compared to the pre-production pellets. We analyzed 43 samples covering nine materials which we grouped according to their feedstock, biodegradability and processing state. We extracted these samples and analyzed the extracts' baseline toxicity, oxidative stress induction and endocrine activity. In addition, we performed non-target high-resolution mass spectrometry (UPLC-QTOF-MS/MS) to characterize the chemicals present in the products.

## 2. Materials and methods

### 2.1. Sample and polymer identification

In total, we selected 43 consumer products and raw materials (pre-production pellets, Table 1). The samples cover 27 bioplastics with the highest market share, including materials that are bio-based and biodegradable (PLA, PHA), petroleum-based and biodegradable (PBS, PBAT) as well as bio-based and not biodegradable (Bio-PE, Bio-PET; European Bioplastics, 2018). In addition, we analyzed 16 plant-based materials (starch, cellulose, bamboo). Thirty-one samples held an inscription to be suitable as food contact materials (FCMs). We acquired raw materials, intermediate and final products from local retailers, online suppliers and at a plastics trade fair. We analyzed the products by Fourier-transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum Two, Waltham, Massachusetts; Fig. S1). The spectra of the samples can be accessed under DOI: 10.5281/zenodo.4004763. Using FTIR, we could not differentiate whether the PE and PET used in the products were made from renewable feedstocks or petroleum. Furthermore, we could not always confirm with certainty the material types indicated by the producer, distributor or vendor due to the absence of openly

available spectral libraries covering bioplastics and plant-based materials or due to products being blends or composites. Thus, we named and categorized the products based on the origin (bio-based or petroleum-based) and biodegradability of their most prominent component labeled on the product or specified by the supplier. Many products are blends of more than one material (see Tab. S1; Peelman et al., 2013), and we obtained only limited information on the formulation of the samples from the suppliers, despite repeated requests. While in the European Union, monomers, catalysts and additives are regulated under REACH and positive lists exist, producers are not required to publicly disclose the exact chemical formulation of their products (Groh et al., 2019).

### 2.2. Sample extraction

To avoid sample contamination, we used glass or polytetrafluoroethylene consumables whenever feasible, rinsed all materials twice with acetone (pico-grade, LGC Standards) and annealed glass items at 200 °C for  $\geq 3$  h. Additionally, we conducted the sample preparation and the bioassays under a laminar flow hood. For sample preparation, the content was removed from packaging samples and the products were rinsed thoroughly with ultrapure water until all residues were removed. Samples were cut with scissors into 0.5–0.8 × 2 cm pieces. While we were aiming at achieving similar surface areas for all samples, these varied due to the different thickness of the samples. Therefore, we decided to extract the same masses. Three grams of each were placed in one or two amber glass vials, depending on their volume. After adding 20 mL methanol (99.9% LC-grade, Sigma-Aldrich), samples were extracted by sonication in an ultrasound bath for 1 h at room temperature. We used methanol because we aimed at maximizing the extraction of chemicals without dissolving the material completely and to be able to compare our results with our previous study on conventional plastics (Zimmermann et al., 2019). The methanol was transferred into clean glass vials and 200  $\mu$ L of the methanol extracts were retained for chemical analysis. After adding 200  $\mu$ L dimethyl sulfoxide (DMSO, Uvasol, Merck) as a keeper, samples were evaporated under a gentle stream of nitrogen to a final volume of 200  $\mu$ L and stored at  $-20$  °C prior to *in vitro* analysis. In order to avoid the loss of compounds, extracts were not filtered and, thus, may contain nano- and microplastics. Four procedural blanks (PB 1–4) consisting of amber glass vials not containing any sample but 20 mL methanol were treated identically to control for a potential contamination. To contextualize the bioassay results, we use "plastic equivalents" in such that "1 mg plastic" implies the toxicity extracted from 1 mg of plastic. Accordingly, 1  $\mu$ L extract corresponds to 15 mg plastic. We here report the masses extracted and applied per well of the respective bioassays.

### 2.3. Bioassays

All bioassays were conducted in 96-well microtiter plates with negative controls (without solvent), solvent controls (DMSO), procedural blanks (PB) and a solvent blank (SB). Samples, solvent controls and blanks were diluted 100-fold (baseline toxicity), 200-fold (oxidative stress response) or 480-fold (endocrine activity) with medium, resulting in a maximum final solvent concentration of 1%, 0.5% or 0.2% (v/v), respectively. Since DMSO did not exhibit any effects compared to negative controls in these concentrations, the results for negative and solvent controls were pooled. In addition, we analyzed solvent blanks (20 mL methanol used for the extraction evaporated to 200  $\mu$ L DMSO) and procedural blanks (PB, treated exactly like the samples but not containing any material). Throughout the experiments, none of the blanks induced toxicity (see Tab. S3–S6). Thus, there was no contamination during sample extraction and analysis. Pooled blanks (control, C) are presented in the bioassay results (Fig. 1, Fig. S2, S4–S8 and S10).

**Baseline toxicity.** The Microtox assay with the bioluminescent

**Table 1**

Bioplastics and plant-based materials analyzed in this study and total number of chemicals features detected by UPLC-QTOF-MS/MS. FCM: Indication that material is suitable for food contact, Type: Raw material (RM), final product (P).

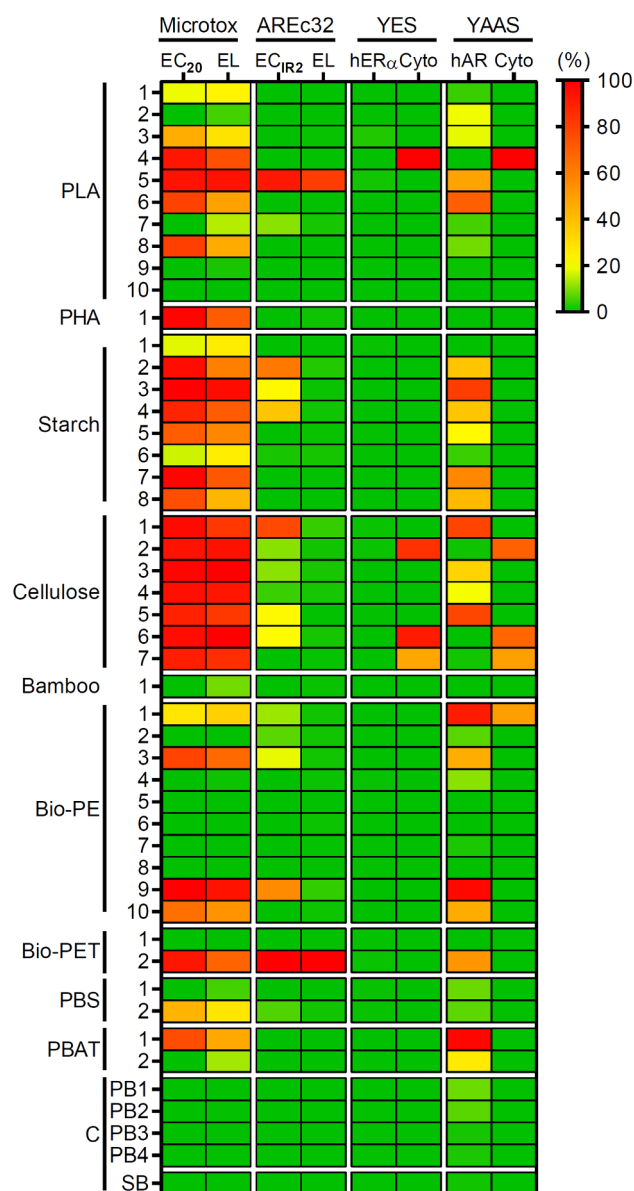
Plastic category	Sample and plastic type	Plastic product	FCM	Type	Number of detected features	
Bio-based, biodegradable	PLA 1	Single-use drinking cup	+	P	3755	
	PLA 2	Disposable cutlery	+	P	3479	
	PLA 3	Film	+	P	8648	
	PLA 4	Food tray	+	P	6465	
	PLA 5	Coffee capsule	+	P	6121	
	PLA 6	Bag for foodstuff	+	P	17,224	
	PLA 7	Single-use bottle	+	P	3002	
	PLA 8	Film		P	10,958	
	PLA 9	Pellet	+	RM	3667	
	PLA 10	Pellet		RM	880	
Petroleum based, biodegradable	PHA 1	Pellet		RM	614	
	PBS 1	Plastic bar		RM	3864	
	PBS 2	Food tray	+	P	10,959	
	PBAT 1	Waste bag	+	P	15,843	
Plant-based	PBAT 2	Pellet	+	RM	9161	
	Starch 1	Disposable cutlery	+	P	1065	
	Starch 2	Bag for foodstuff	+	P	18,198	
	Starch 3	Film		P	15,770	
	Starch 4	Film	+	P	16,857	
	Starch 5	Pellet	+	RM	9118	
	Starch 6	Pellet	+	RM	8325	
	Starch 7	Waste bag	—	P	20,965	
	Starch 8	Film		P	11,901	
	Cellulose 1	Tea bag wrapping	+	P	14,456	
	Cellulose 2	Chocolate wrapping	+	P	3378	
	Cellulose 3	Cigarette filter	—	P	15,719	
	Cellulose 4	Pellet	+	RM	2953	
	Cellulose 5	Bag for foodstuff	+	P	20,416	
	Cellulose 6	Bag for foodstuff	+	P	14,031	
	Cellulose 7	Bag for foodstuff	+	P	17,495	
	Bamboo 1	Reusable coffee cup	+	P	5426	
	Bio-based, non-biodegradable	Bio-PE 1	Bag for foodstuff	+	P	5272
		Bio-PE 2	Wine closure	+	P	1629
		Bio-PE 3	Bag for foodstuff	+	P	n.a. <sup>a</sup>
Bio-PE 4		Pellet		RM	819	
Bio-PE 5		Food tray	+	P	290	
Bio-PE 6		Film		P	928	
Bio-PE 7		Wine closure	+	P	947	
Bio-PE 8		Pellet		RM	186	
Bio-PE 9		Bag for foodstuff	+	P	19,028	
Bio-PE 10		Film	+	P	13,381	
Bio-PET 1		Reusable bottle	+	P	390	
Bio-PET 2		Box		P	5625	

Note: <sup>a</sup> n.a., not analyzed.

bacterium *Aliivibrio fischeri* was performed according to an ISO guideline (ISO 11348-3, 2017) miniaturized to a 96-well plate format (Escher et al., 2008). In brief, extracts and controls including the reference compound 3,5-dichlorophenol (Tab. S2, Fig. S3) were analyzed in serial dilutions (1:2 in saline buffer). For extracts, these dilutions correspond to 0.18–22.5 mg plastic. Fifty  $\mu\text{L}$  of *A. fischeri* suspension was added to 100  $\mu\text{L}$  diluted sample. Luminescence was measured prior to and 30 min after sample addition using a Spark 10 M microplate reader (Tecan, Crailsheim, Germany). In accordance with the ISO guideline (ISO 11348-3, 2017), the results were corrected for the luminescence in the blanks (empty wells) and for the change in luminescence in negative controls over 30 min, resulting in a relative luminescence inhibition (%). Dose-response relationships were derived for each sample using a four-parameter logistic model with the lower and upper plateau constrained to 0 and 100% luminescence inhibition, respectively. Results from two to five independent experiments with two technical replicates each are expressed as effect concentration ( $\text{EC}_{20} \pm \text{SEM}$ , mass of plastic well<sup>-1</sup> inducing a 20% luminescence inhibition) and mean effect size  $\pm \text{SEM}$  (luminescence inhibition induced by 22.5 mg plastic well<sup>-1</sup>). In case an  $\text{EC}_{20}$  could not be derived, we used an  $\text{EC}_{20}$  of 25 mg plastic well<sup>-1</sup> to visualize the data, indicating that the  $\text{EC}_{20}$  is larger than the highest analyzed concentration.

**Oxidative stress response.** We used the AREc32 assay to investigate

the induction of an oxidative stress response in the Nrf2/ARE pathway (Wang et al., 2006). The AREc32 cell line was obtained from Signosis Inc. (catalog number: SL-0010-NP, Santa Clara, CA, USA). The assay was performed as described previously by Völker et al. (2017), with minor modifications. In brief, 12,000 cells well<sup>-1</sup> were seeded in 96-well plates. After 24 h, 100  $\mu\text{L}$  medium well<sup>-1</sup> was replaced by medium containing serial dilutions (1:2 in medium) of the samples (0.06–7.5 mg plastic well<sup>-1</sup>) or the reference compound *tert*-butylhydroquinone (t-BHT, Tab. S2, Fig. S3). After 24 h, cell viability and luciferase activity were determined. Cytotoxicity was determined via the metabolic reduction of resazurin according to Palomino et al. (2002) with minor modifications. Resazurin sodium salt was dissolved at 0.01% (w/v) in phosphate buffer saline (PBS) and filtered (0.2  $\mu\text{m}$ ). Thirty  $\mu\text{L}$  resazurin solution was added to each well, incubated for 5.5 h and photometrically measured at 570 and 600 nm (Spark 10 M, Tecan, Crailsheim, Germany). Based on the absorbance of resazurin and resorufin (reduced from resazurin by living cells), the percentage of living cells was calculated. Extracts were considered cytotoxic if they reduced the cell number by > 10% compared to the control. The luciferase activity was determined immediately after adding 100  $\mu\text{L}$  0.015% w/v beetle luciferin potassium salt (Promega, E1601) using a Spark 10 M microplate reader. Each sample was analyzed in two to four independent experiments with duplicates each. In order to control for the variability



**Fig. 1.** Toxicological signature of bioplastics and plant-based materials based on baseline toxicity (Microtox), oxidative stress response (AREc32) as well as estrogenic (YES) and antiandrogenic activities (YAAS). The results are presented as gradient from 0 (green) to 100% (red). The endocrine activities were used as such while the other results were normalized to the lowest and highest effect observed for the respective endpoint. For AREc32 ELs, the highest non-cytotoxic concentrations (Tab. S4) were used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between experiments as well as 96-well plates, t-BHT was analyzed on each plate. We only considered those plates on which the t-BHT dose–response relationship was within the 95% confidence interval of the previously verified full-dose response relationship (see Fig. S3). We excluded the concentrations that were cytotoxic in the respective experiment and replicate and derived dose–response relationships for the induction ratios (IR) using a four-parameter logistic model (lower plateau constrained to 1) to interpolate the plastic mass producing an IR of 2 over the control (EC<sub>IR2</sub>). In case an EC<sub>IR2</sub> could not be derived, we used an EC<sub>IR2</sub> of 8 mg plastic well<sup>-1</sup> to visualize the data, indicating that the EC<sub>IR2</sub> is larger than the highest analyzed concentration. The IR

at the highest non-cytotoxic concentration (across all experiments) is also reported.

**Endocrine activity.** We used yeast-based reporter-gene assays to investigate the induction of agonistic activity at the human estrogen receptor  $\alpha$  (hER $\alpha$ ; Routledge and Sumpter, 1996) and antagonistic activity at the human androgen receptor (hAR; Sohoni, 1998). The Yeast Estrogen Screen (YES) and the Yeast Antiandrogen Screen (YAAS) were performed as previously described with minor modification (Wagner and Oehlmann, 2009). In brief, samples were diluted 480-fold in medium resulting in a final sample concentration of 3.75 mg plastic well<sup>-1</sup>. Samples that induced  $\geq 20\%$  cytotoxicity were excluded and re-analyzed in additional 1:2 serial dilutions (lowest concentration: PLA 4/Cellulose 2: 7.3  $\mu\text{g}$  plastic well<sup>-1</sup>, Cellulose 6/7: 234  $\mu\text{g}$  well<sup>-1</sup> and in the YAAS additionally: Bio-PE 1: 469  $\mu\text{g}$  well<sup>-1</sup>). Additionally, further samples with antiandrogenic effects were also diluted in a 1:2 series (lowest concentration: PLA 5: 7.3  $\mu\text{g}$  plastic well<sup>-1</sup>, Bio-PE 9/PBAT 1: 234  $\mu\text{g}$  plastic well<sup>-1</sup> and PLA 6/Starch 3/Cellulose 1/Bio-PET 2: 469  $\mu\text{g}$  plastic well<sup>-1</sup>). Starch 7 and Cellulose 5 were not analyzed in dilutions since their sample volume was restricted. 17 $\beta$ -estradiol and flutamide served as reference compounds for the YES and YAAS, respectively (Tab. S2, Fig. S3). To determine the antagonistic activity in the YAAS, 10 nmol L<sup>-1</sup> testosterone, inducing  $\sim 75\%$  receptor activation, was added. The initial cell density was adjusted to formazin attenuation units (FAU) of 25 for the YES and 100 for the YAAS. After 20 h incubation, we determined the cell density as absorbance at 595 nm on a Spark 10 M instrument. After transferring 30  $\mu\text{L}$  well<sup>-1</sup> to a new 96-well plate, 50  $\mu\text{L}$  lacZ buffer containing 1.5 mmol L<sup>-1</sup> 4-methylumbelliferyl  $\beta$ -D-galactopyranoside (MUG, Merck, CAS 6160-78-7) and 1 mmol L<sup>-1</sup> dithiothreitol (Sigma-Aldrich, CAS 3483-12-3) was added. The fluorescence (excitation: 360 nm, emission: 465 nm) was determined after 40 min incubation at 30  $^{\circ}\text{C}$  using a Spark 10 M instrument. We also analyzed all samples for auto-fluorescence prior to the MUG addition and did not observe any. In the YES, all samples were analyzed in two (exception PLA 3: three, Cellulose 2: four) and in the YAAS in two to six independent experiments with eight replicates, each.

Data was processed as previously described to derive the relative cytotoxicity as well as relative estrogenic and antiandrogenic activities (Völker et al., 2016). The limit of detection (LOD) of each experiment was calculated as three times the standard deviation (SD) of pooled negative and solvent controls. Effects  $>$  LOD were considered significant. Dose–response relationships for cytotoxicity and relative endocrine activity were calculated using a four-parameter logistic function constrained to bottom level of zero (0% cytotoxicity/activity) and for cytotoxicity also a top of 100%. The respective plastic equivalents inducing 20% cytotoxicity (EC<sub>20</sub>) were interpolated from the dose–response curves. For the antiandrogenic activity, the EC<sub>50</sub> was used. To ensure comparability of independent experiments only those experiments were considered in which the dose–response relationship of the reference compound had a  $r^2 > 0.9$ , a minimal relative luminescence unit  $< 4500$  and a maximal  $> 50,000$  as well as an EC<sub>50</sub> of  $2\text{--}30 \times 10^{-11}$  mol L<sup>-1</sup> 17 $\beta$ -estradiol (YES) or  $1\text{--}4.8 \times 10^{-5}$  mol L<sup>-1</sup> flutamide (YAAS, Tab. S2). The mean EC<sub>50</sub> of 17 $\beta$ -estradiol and flutamide analyzed in each experiment (95% confidence intervals) were  $1.26 \times 10^{-10}$  mol L<sup>-1</sup> ( $0.23\text{--}2.29 \times 10^{-10}$ ) and  $1.88 \times 10^{-5}$  mol L<sup>-1</sup> ( $1.21\text{--}2.56 \times 10^{-5}$ ), respectively.

#### 2.4. Chemical analysis

Non-target screening of the chemicals extracted from the samples was conducted using ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry/mass spectrometry (UPLC-QTOF-MS/MS) on a Acquity UPLC Waters Liquid Chromatography system (Waters Norge, Oslo, Norway) coupled to a SYNAPT G2-S mass spectrometer (Waters Norge, Oslo, Norway) in positive ionization mode. Two  $\mu\text{L}$  methanol extracts (0.15 mg plastic



$\mu\text{L}^{-1}$ ) were injected onto an Waters C18 guard column coupled to an Acquity UPLC BEH C18 column (130 Å, 1.7  $\mu\text{m}$ , 2.1  $\times$  150 mm, Waters) with a column temperature of 40 °C. The LC flow rate was 0.2 mL  $\text{min}^{-1}$  using H<sub>2</sub>O with 0.1% formic acid and methanol with 0.1% formic acid as mobile phases A and B, respectively. The gradient started with 80:20% A:B for 0.5 min, then increased to 40:60% at 4.5 min and to 0:100% at 35.5 min. 100% B was maintained until 38.5 min, returned to 20:80% at 39.5 min and equilibrated for 2 min prior to the next injection. The heated electrospray ionization source (positive mode) had a capillary temperature of 120 °C with a spray voltage of 2.5 kV and a sampling cone voltage of 30 V. The desolvation gas flow was 800 L  $\text{h}^{-1}$ . The mass spectrometer was run in full scan (50–1200 Da) at a resolution of 20,000 with a data-independent MS<sup>E</sup> Continuum acquisition with a low collision energy (4 eV) and a high collision energy ramp (15–45 eV). Each sample was analyzed once. LC blanks (methanol) were analyzed approximately after every seventh sample to exclude column contamination. We did not analyze Bio-PE 3 because it contained particulate matter. The mass spectral data of all samples can be accessed under DOI: 10.5281/zenodo.4004763.

## 2.5. Analysis of chemical data and compound identification

We used Progenesis QI (version 2.3, Nonlinear Dynamics, Newcastle upon Tyne, UK) to analyze the UPLC-QTOF-MS/MS data. In brief, we imported all raw data files (blanks and samples), enabled the search for common adducts (M + H, M + Na, M + H-H<sub>2</sub>O, 2 M + Na, 2 M + H, M + H-2H<sub>2</sub>O, M + CH<sub>3</sub>OH + H, M + 2H) and calibrated the *m/z* of all runs using the internal lock mass of leucine enkephalin (556.2766 *m/z*). We automatically aligned the retention times of all runs and performed the peak picking (automatic sensitivity, no predefined peak width).

We exported the resulting feature list to Microsoft Excel for Mac (version 16.35) and compared the maximum raw abundance of each feature in the blanks (*n* = 14) to the raw abundance of the same feature in the individual samples. We filtered for features that were not present in the blanks but in the sample or had a tenfold higher abundance in the sample than in the blank. Based on those results, we identified the ten features that had the highest abundance in each sample as well as the features that were most prevalent across all samples (present in  $\geq 30$  samples). In addition, we used the features present in at least one sample per material to compare the different materials and identify those present in more than one material. Using Progenesis QI, we tentatively identified these features by searching all available data sources in ChemSpider with a precursor tolerance of 5 ppm, a fragment tolerance of 10 ppm and a 50% isotope similarity filter. In addition, we performed theoretical fragmentations of the ChemSpider results using the MetaScope algorithm. For each feature, we inspected manually at least the 25 hits with the highest scores and selected the compound identity based on score and plausibility (e.g., by excluding rare elements or salts and focusing on formulas containing C, H, O, N, only). For accepted compounds with a match score > 50, we also performed a PubChem search to retrieve additional information on the use and functionality.

## 2.6. Statistical analysis of bioassay data

We used GraphPad Prism 5 and 8 (GraphPad Software, San Diego, CA) for nonlinear regressions and statistical analyses. To compare two treatments, we used unpaired t-tests for parametric and Mann-Whitney tests for not normally distributed data. A *p* < 0.05 was considered statistically significant. We performed cluster analyses to compare the toxicological (Microtox EC<sub>20</sub>, AREc32 EC<sub>IR2</sub>, and YES/YAAS relative activity) and chemical signatures of the samples. For the latter, we generated a joint peak list containing the abundances of all masses detected in the samples but not in the blanks (see 2.5). We calculated the Euclidean distance between samples and clustered them hierarchically using the “complete linkage” method with the “dist” and

“hclust” functions in R (RStudio, 2016).

## 3. Results

### 3.1. Baseline toxicity

The bioluminescence inhibition of *Aliivibrio fischeri* is an indicator for baseline toxicity that is more sensitive than other endpoints for unspecific toxicity, such as cytotoxicity in mammalian cells (Neale et al., 2012). Two thirds (67%) of the 43 extracts (Fig. 1, S4 and S5, Tab. S3) induced baseline toxicity. All cellulose-based and starch-based samples as well as the PHA sample inhibited bioluminescence, mostly with a high potency (low EC<sub>20</sub>) and effect level. The bamboo product did not have any effect in the Microtox assay. The baseline toxicity triggered by the other materials varied with the sample: Six out of ten PLA samples, four out of ten Bio-PE as well as one out of two Bio-PET, PBS and PBAT samples, each, inhibited the bioluminescence.

### 3.2. Oxidative stress response

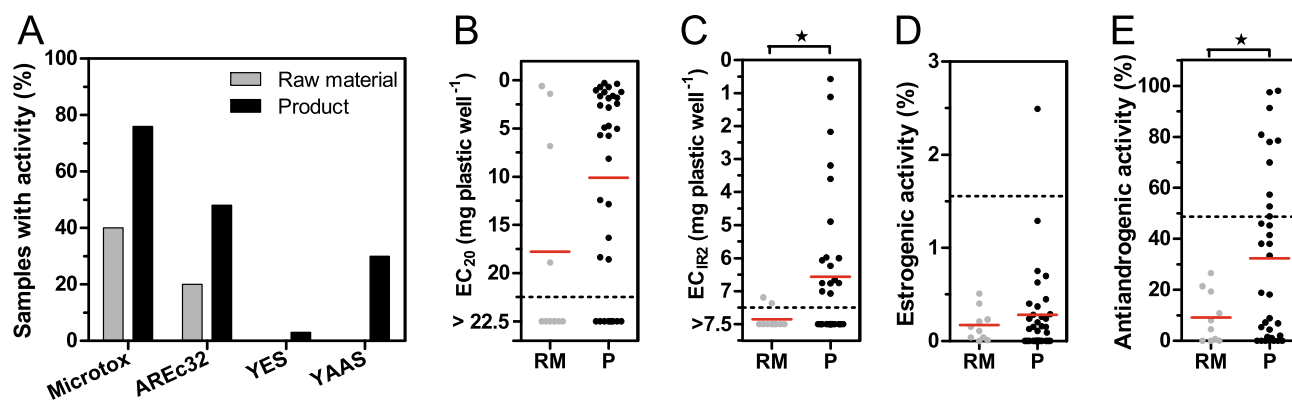
In the AREc32 assay, human MCF-7 cells are used to investigate the induction of the Nrf2-ARE regulated oxidative stress response (Wang et al., 2006). Eighteen out of 43 samples (42%) activated this pathway (Fig. 1, S6 and S7, Tab. S4). The Bio-PET 2 extract was most potent (EC<sub>IR2</sub> = 0.58 mg plastic well<sup>-1</sup>) and had the highest effect level (IR = 64.8), followed by PLA 5 (EC<sub>IR2</sub> = 1.12 mg plastic well<sup>-1</sup>, IR = 52.5). In addition, six out of seven cellulose-based, four out of eight starch-based, four out of ten Bio-PE, two out of ten PLA and one out of two PBS samples activated the oxidative stress response. However, for most of these samples, effects strongly varied between independent experiments. For example, we measured the strongest variation for Bio-PE 9 with one replicate having an EC<sub>IR2</sub> of 0.54 and another of > 7.5 mg plastic well<sup>-1</sup>. None of the PHA, PBAT, bamboo-based samples induced an effect.

### 3.3. Endocrine activity

To investigate whether products contain estrogen receptor agonists or androgen receptor antagonists, we analyzed the samples in yeast-based reporter gene assays. PLA 3 was the only extract that activated the human estrogen receptor  $\alpha$  above the LOD (1.56%) with a relative activity of 2.49% at 3.75 mg plastic well<sup>-1</sup> (Fig. 1, S8, Tab. S5). Four samples (PLA 4, Cellulose 2, 6, 7) were cytotoxic and inactive when analyzed in dilutions (Fig. S9). Compared to the estrogenicity, the extracts' antiandrogenic activity (LOD = 48.6%) was more pronounced, with ten out of 43 samples inhibiting the androgen receptor by 49–98% at the highest non-cytotoxic concentration (Fig. 1, S10, Tab. S5). Here, PBAT 1 (98.0%), Bio-PE 9 (97.4%) and Bio-PE 1 (91.3%) induced the strongest effects. Additionally, two PLA, starch and cellulose samples, each, as well as one Bio-PET extract were antiandrogenic. We also analyzed the dose–response relationships of selected samples that were either antiandrogenic or cytotoxic. Here, PBAT 1 and Bio-PE 9 were most potent with EC<sub>50</sub> values in the YAAS of 0.40 and 0.39 mg material extracted, respectively (Fig. S11).

### 3.4. Toxicological signatures of plastics

The toxicological signatures highlight that the chemicals extracted from cellulose and starch samples affected most endpoints, especially baseline toxicity (Fig. 1). In contrast, the bamboo sample and Bio-PE samples contained the lowest toxicity. Nonetheless, four out of ten PE samples had an effect in at least one bioassay, with Bio-PE 9 being very antiandrogenic. The toxicological signatures of PLA extracts were more heterogeneous with PLA 4 and 5 inducing the highest and broadest toxicological response. We observed a similarly heterogeneous picture for the other materials. For example, Bio-PET, PBS and PBAT



**Fig. 2.** Toxicity of extracts from raw materials (RM,  $n = 10$ ) compared to final products (P,  $n = 33$ ) with regards to the percentage of active samples (A) and the mean effect strengths for baseline toxicity (B, Microtox), oxidative stress response (C, AREc32), estrogenic (D, YES) and antiandrogenic activity (E, YAAS). It remains unknown whether the final products were produced from the analyzed raw materials. Each dot represents one sample and red lines the mean. For D and E, effects are shown for 3.75 mg plastic well<sup>-1</sup> or, if cytotoxic, for the highest non-cytotoxic concentration (Tab. S5). \*  $p < 0.05$ , unpaired Mann-Whitney test for (C) and unpaired  $t$ -test for (E), dotted lines = highest analyzed concentration (B, C) or limit of detection (D, E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

comprising one toxic and one non-toxic sample each.

We performed a cluster analysis to test the hypothesis that the material predicts the toxicity of a sample. The samples clustered in four main groups (Fig. S12). The group 2 in the tree includes the samples with the highest toxicity inducing three endpoints. Here, the effect strength was high for at least two and medium for a third endpoint. Groups 1 and 3 cover samples that induced a medium toxicity. The samples in the first group either affected more endpoints or had a higher effect strengths compared to those in the third group. Group 4 comprises the samples with the lowest toxicity affecting no or one endpoint. There was no specific clustering of samples according to their material indicating that the polymer type is not predictive for the toxicity of these materials.

### 3.5. Comparison of raw materials and final products

To investigate whether final products contain a higher chemical toxicity than the raw materials, we pooled the data from the 33 final products and the ten pre-production pellets. Across all endpoints, more final products induced toxicity compared to the raw materials (Fig. 2). For unspecific endpoints, the percentage of final products having an effect was double that of raw materials, with 78 vs. 40% of the samples inducing baseline toxicity and 48 vs. 20% of samples inducing an oxidative stress response. None of the raw materials contained estrogen-like or antiandrogenic chemicals, whereas 30% of the final products were antiandrogenic. Regarding the mean effect level, final products induced stronger toxicity on all endpoints. However, two raw materials induced a baseline toxicity that was as high as that of the most toxic final products.

### 3.6. Comparison of bioplastics and plant-based materials with conventional plastics

To analyze whether bio-based and/or biodegradable materials contain chemicals that are less toxic than those in conventional (petroleum-based, non-biodegradable) plastics, we pooled the data from all samples analyzed here and compared it to the data from our previous study in which we tested 30 conventional plastics in exactly the same way as in the present study (Zimmermann et al., 2019). The proportion of samples inducing toxicity was the same for the bio-based/biodegradable materials as for the conventional plastics. A slightly higher percentage of bioplastics and plant-based materials compared to conventional plastics induced baseline toxicity and a slightly higher percentage of conventional plastics had an endocrine activity (Fig. 3). The

mean effect strengths of bioplastics and plant-based materials were comparable with conventional plastics across all endpoints, except for estrogenicity which was induced significantly stronger by conventional plastic than bio-based/biodegradable materials. However, this difference was mainly driven by one PVC extract with a relative estrogenic activity of 27.1%.

Comparing petroleum-based plastics with their direct bio-based counterparts, for PE a higher number of bio-based samples induced oxidative stress. However, it was a petroleum-based PE that was most effective (LDPE 4:  $EC_{100} = 0.48$  mg plastic well<sup>-1</sup>). More bio-based PE extracts inhibited the androgen receptor and did so with a higher efficiency (up to 97.4%). Interestingly, none of the five conventional PET extracts induced relevant toxicity but one out of the two Bio-PET samples did.

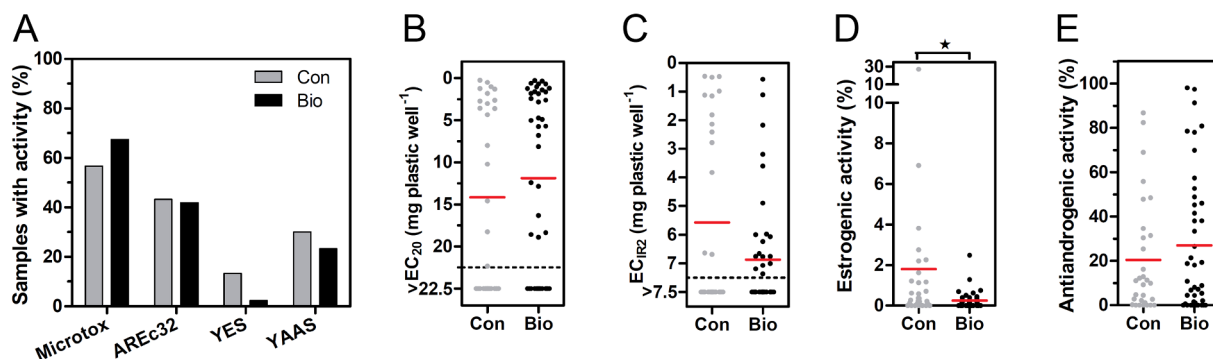
### 3.7. Chemical features

In total, we detected 51,677 chemical features across the 14 blanks and the 42 samples. Filtering for features that had at least a tenfold higher abundance in samples compared to the blanks, resulted in a total of 41,395 features in all samples. The individual samples contained between 186 and 20,965 features (Tab. 1). Thirty-four samples had > 1000 features each with Starch 7 (20,965 features), Cellulose 5 (20,416) and Bio-PE 9 (19,028) containing the highest numbers. On the other end of the spectrum, Bio-PE 8 (186), Bio-PE 5 (290) and Bio-PET 1 (390) contained the least chemical features.

### 3.8. Chemical similarity of materials

We compared the similarity of chemical features within and between materials. In total, between 5811 and 31,727 different features were detected per material (of which at least two products were analyzed). When investigating whether features were shared among multiple samples per material, it became clear that most were unique to one sample (Fig. 4A). For instance, about half of all features detected in PLA and Bio-PE were present in only one but not the other samples of the same material. This was less pronounced for starch and cellulose with about 30% of all features being unique to one sample per material. Here, a higher number of features was detected in multiple samples. For instance, 11% of all features were shared by five samples/material. Less than 1.1% of all features detected in a material was present in all samples of that material, corresponding to 285 features for PLA, 110 for starch, 257 for cellulose and 0 for Bio-PE.

Taking a similar approach to compare the features present across



**Fig. 3.** Toxicity of extracts from conventional, petroleum-based (Con,  $n = 30$ ) compared to bioplastics and plant-based materials (Bio,  $n = 43$ ) with regards to the percentage of active samples (A) and the mean effect strengths for baseline toxicity (B, Microtox), oxidative stress response (C, AREC32), estrogenic (D, YES) and antiandrogenic activity (E, YAAS). Each dot represents one sample and red lines the mean. For D and E, effects are shown for 3.75 mg plastic well<sup>-1</sup> or, if cytotoxic, for the highest non-cytotoxic concentration (Tab. S5). \*  $p < 0.05$ , unpaired Mann-Whitney test, dotted lines = highest analyzed concentration. Toxicity data for conventional materials are taken from Zimmermann et al. (2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

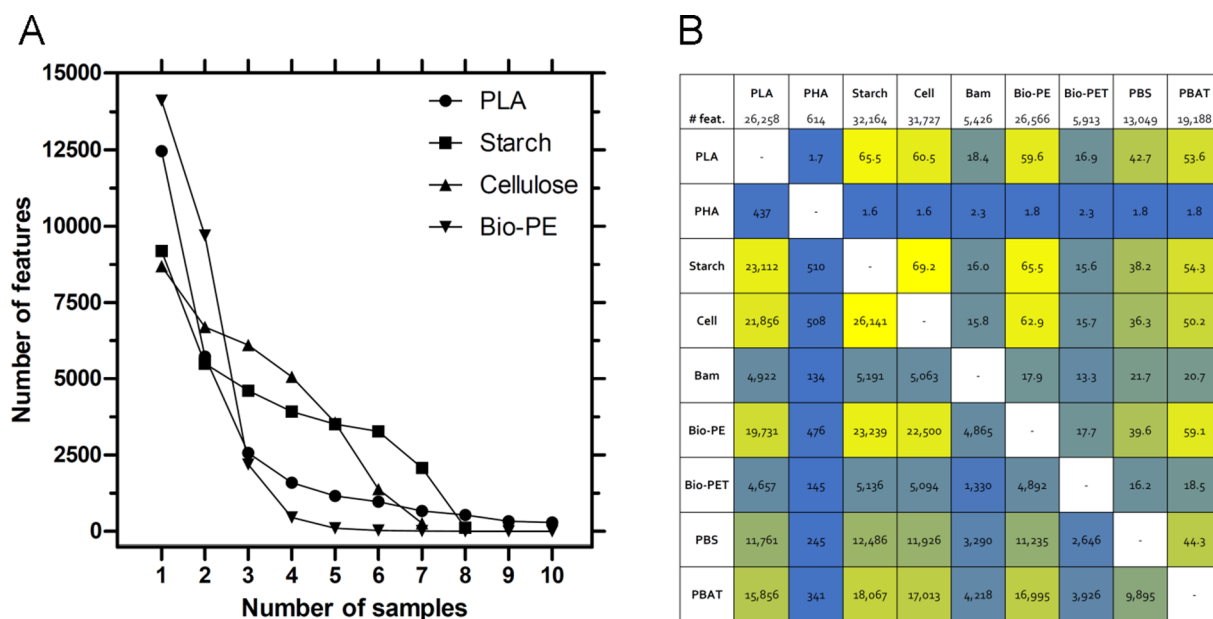
different materials, we found that a total of 37 features was present in all materials (i.e., in at least one sample per material). Whereas materials with few features (PHA, bamboo, Bio-PET) shared little chemical similarity with the other materials (Fig. 4B), PLA, starch, cellulose, Bio-PE, PBS and PBAT shared more than one third of all features. PLA and starch, starch and cellulose, starch and Bio-PE as well as cellulose and Bio-PE shared at least two thirds of all detected features (each combination shared > 20,000 features). Further, a cluster analysis using the abundance of all features did not return distinct clusters for the materials (Fig. S13). In some cases, two samples from the same material clustered closely, implying similar chemical signatures. However, in most cases the similarity between materials was higher than within materials.

### 3.9. Tentatively identified compounds

We tentatively identified the most prevalent features across all samples (i.e., most often detected) and the most abundant features in

each sample (i.e., highest intensity). In total, 42 out of the 45 chemical features present in at least 30 samples were identified by the MetaScope algorithm (Tab. S6). The most prevalent feature ( $m/z$  641.6915, charge 2+) was detected in 37 samples but remained unidentified. The second most prevalent feature (in 35 samples) is a benzofuran carboxylate with a relatively high match score. However, upon comparison with the PLA oligomers described by Ubeda et al. (2019) this feature appears to be a cyclic lactic acid oligomer ( $(C_3H_4O_2)_n$  with  $n = 6$ ). Interestingly, two other compounds also share spectral similarities with PLA oligomers (Tab. S6). Three compounds had a match score > 50: 4-Amino-6-(2-furyl)-2-[2-(4-morpholinyl)-2-oxoethyl]-3(2H)-pyridazinone, (2Z)-4-Methyl-2-pentene-2,3,4-tricarboxylic acid and 2,3,4-Tri-O-acetyl-6-O-(2-methoxy-2-oxoethyl)-alpha-D-galactopyranose (present in 30 samples, each). PubChem did not contain any relevant information on the origin or use of these chemicals.

The ten most abundant features per sample comprised 294 different features indicating some overlap between samples. Out of these, we tentatively identified 271 compounds (Tab. S7). Twenty-six had a score



**Fig. 4.** Number of chemical features plotted according to the number of samples per material it is detected in (A) and number of features shared between materials (B). In B, features are considered that have been detected in at least one sample per material (sum given as # feat.). The lower left section represents the number of shared features, the upper right section their percentage of all features detected in the combination of materials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of > 50, including N,N'-1,4-Butanediyldihexadecanamide (used as plasticizer and in coatings/paints), 2-Ethoxyethyl hexadecyl (2E)-2-butenedioate (a fumaric acid that may be used as monomer; ECHA, 2020), 2,2'-(Tridecylimino)diethanol (CAS 18312-57-7, a surface active agent that migrates from PP packaging; Aznar et al., 2012). Some of the remaining compounds may be of natural origin (microbial, fungal or plant) but did not have relevant information regarding their origin/use or their identification was implausible. Other notable compounds with a lower match score are the lubricant and plastic additive N,N'-ethylenebis(palmitamide) (CAS 5518-18-3, detected in PLA; NCBI, 2020), the NIAS 1,6,13,18-tetraoxacyclotetracosane-2,5,14,17-tetrone (CAS 141850-18-2, detected in PLA, starch, Bio-PE and PBAT), the plastic additive erucamide (CAS 112-84-5, detected in starch, cellulose and Bio-PE), the antioxidant Irganox 1076 (CAS 2082-79-3, in Bio-PE), and the antioxidant degradation product tris(2-nonylphenyl) phosphate (CAS 26569-53-9, in Bio-PE). Interestingly, some of the top 10 compounds were not unique to one but also detected in other samples in high abundances including ones made of different materials (Tab. S7). As in case of the most prevalent features, some of the top 10 abundant features in PLA shared similarities with PLA oligomers. Four of those were probably cyclic lactic acid oligomers with  $n = 6-9$  based on their mass spectra (Ubeda et al., 2019).

Regarding the features that were present in all material types, we tentatively identified 30 out of the 37 (Tab. S8). The seven features with a match of > 50% were 3-Pyridinylmethyl {4-[5-({4-[2-(4-morpholinyl)ethoxy]benzoyl)amino)-1H-pyrazol-3-yl]benzyl}carbamate, 1-(Bicyclo[2.2.1]hept-2-yl)-3-(3-chloro-4-pyridinyl)acetone, methyl (2E,4E,6S,8E,13R)-13-acetoxy-6-hydroxy-2,4,8-tetradecatrienoate, N-[4-(2-Furyl)-4-hydroxy-2-butanyl]-1-(3-methylbutyl)-1H-1,2,3-triazole-4-carboxamide, S-[(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-yl]methanesulfonothioate, 1-[4-Hydrazino-6-(1H-1,2,4-triazol-1-yl)-1,3,5-triazin-2-yl]-3-pyrrolidinecarboxamide and (2S,3R,4S,5S,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-2-[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]tetrahydro-2H-pyran-2-carboxamide. PubChem did not contain any relevant information regarding their origin, function or use. Interestingly, the tentatively identified compounds did not include per- and polyfluoroalkyl substances (PFAS).

## 4. Discussion

### 4.1. Bioplastics and plant-based materials contain chemicals inducing *in vitro* toxicity

Bioplastics and plant-based materials are promoted as more sustainable alternative to conventional, petroleum-based non-biodegradable plastics (Lambert and Wagner, 2017). However, currently we do not know whether they also represent a safer alternative with regards to the chemicals they contain, including the hazard and human exposure to these compounds. While knowledge on both is required to arrive at a risk-based assessment, we focus on the toxicity in this study. We extracted the materials as a worst-case scenario to generate first insights into the hazard of the mixture of extractable compounds. Using this approach, we demonstrate that out of the 43 products 29 contained chemicals that induced baseline toxicity, 18 that induced oxidative stress, 10 antiandrogenicity and one estrogenicity. This demonstrates that a range of bio-based and/or biodegradable materials, most of them used as FCMs, contain chemicals that are toxic *in vitro*. While we cannot rule out the presence of nano- and microplastics in the samples, their concentration would have been low due to the dilution in the bioassays. Thus, we believe that the observed toxicities are largely caused by the extracted chemicals.

While a systematic assessment is currently missing, previous research also reported *in vitro* toxicity of bioplastics and other bio-based materials. As an example, cellulose-based materials induced cytotoxicity in mouse fibroblasts (Dang et al., 1996). Up to date, research has mainly focused on the unspecific toxicity of PLA. Accordingly,

chemicals leaching from different PLA materials used for medical implants inhibited bacterial bioluminescence (Ramot et al., 2016; Taylor et al., 1994) whereas migrates of PLA-clay nanocomposites used in food packaging were not cytotoxic in human cell lines (Maisanaba et al., 2014). This product-dependent variation of toxicity, even if made of the same material type, corresponds to our findings. For instance, a coffee capsule (PLA 5) but not a single-use bottle (PLA 7) induced *in vitro* toxicity.

Since bioplastics and plant-based materials are often applied in agriculture and horticulture (European Bioplastics, 2018), many studies investigate their *in vivo* toxicity, especially with regards to terrestrial ecosystems. Here, aqueous extracts of pure PLA and PLA-nanoclay induced genotoxicity in the onion *Allium cepa* (Souza et al., 2013). Phytotoxic effects were also observed for leachates of starch-based bags affecting plant germination (Balestri et al., 2019) or whole coastal dune vegetations (Menicagli et al., 2019). Studies comparing different materials indicated a material-dependent toxicity of biodegradable materials used in agriculture in plants (Serrano-Ruiz et al., 2018) and soil bacteria (Adhikari et al., 2016). For instance, PLA but not PBS and PBS-starch affected nitrogen circulation activity of soil bacteria (Adhikari et al., 2016). Studies on the toxicity of polyhydroxybutyrate-based materials (PHB) are currently limited to freshwater species. PHB and PBAT leachates reduced the survival of *Daphnia magna* already after 48 h of exposure (Göttermann et al., 2015).

While previous reports are sporadic and predominately focus on PLA, our results imply that chemicals inducing unspecific toxicity are prevalent in all types of bio-based and/or biodegradable products, especially in those made of the natural polymers starch and cellulose. Our results also indicate that these materials contain endocrine disrupting chemicals, with antiandrogenicity being more frequent and potent than estrogenicity. These finding, along with the absence of systematic research, stress that analyzing the chemical toxicity of bioplastics and plant-based materials, especially of materials other than PLA, should be prioritized in future research. This can be achieved by combining bioassays with analytical chemistry (Bergmann et al., 2020; Groh and Muncke, 2017; Veyrand et al., 2017) and embedded in a green chemistry approach that aims in avoiding the use and generation of hazardous substances. As an example, Bandyopadhyay-Ghosh et al. (2018) synthesized a novel polysaccharide biopolymer that did not induce baseline toxicity or genotoxicity.

### 4.2. Bioplastics and plant-based materials contain a complex mixture of chemicals

Using a non-target screening with UPLC-QTOF-MS, we detected 41,395 chemical features across 42 samples and 186–20,965 features in the individual samples. While products made of starch and cellulose contained the highest number of features (typically > 10,000), the number of substances generally varied from product to product. Most samples (80%) contained more than 1000 chemical features, illustrating the large number and variety of low molecular weight chemicals present in bio-based and/or biodegradable products.

Only few other studies apply non-target analysis to examine compounds in conventional plastics or their “biological” counterparts and the number of detected features is hardly ever reported. As an exception, Aznar et al. (2019) detected 37 non-volatile chemicals in pellets and films made from a PLA/Bio-PE blend using UPLC-QTOF-MS. Bradley (2010) performed a more comprehensive migration study of 13 starch, cellulose, PLA, cassava and bagasse samples and detected up to 32 and 29 compounds using GC-MS and LC-TOF-MS, respectively. Using other instruments and settings, those studies cannot directly be compared to ours. Thus, we probably detected more compounds because we used a different data analysis strategy and considered all features present in the samples with an at least 10-fold higher abundance than in the blanks. Furthermore, the choice of extraction technique (e.g., type of solvent, temperature and duration) will affect the



composition of detected chemicals.

In any case, our results clearly show that bioplastics and similar plastic alternatives contain a strikingly high number and variety of chemicals. While not all of these are relevant for human exposure or the environment, this highlights the challenges we face when aiming to assess the chemical composition and safety of plastics and other synthetic materials, especially when dealing with FCMs (Muncke et al., 2020).

#### 4.3. Chemicals present in bioplastics and plant-based materials

As the non-target analysis resulted in a large number of chemical features, we focused on identifying the compounds that were most prevalent across samples and materials as well as the ten compounds with the highest abundance in individual samples. The *in silico* fragmentation of all corresponding candidates from PubChem resulted in the tentative identification of circa 94% of these chemical features. While this appears promising, care should be taken when interpreting the results. As an example, some of the most prevalent and abundant features in PLA were probably oligomers of lactic acid (Ubeda et al., 2019) and not the compounds identified by the MetaScope algorithm. Likewise, some of the features were identified as pharmaceuticals or natural products which we do not expect to occur in our samples. This highlights the challenges of unknown analysis: General chemical databases often do not cover chemicals used in the manufacture of (semi) synthetic polymers making a query of empirical or theoretical spectra difficult.

These limitations notwithstanding, we tentatively identified a range of plausible compounds in bioplastics and plant-based materials. We found a number of plastic additives, including butanediyldihexadecanamide, ethylenebis(palmitamide), erucamide and Irganox 1076 as well as NIAS, including tetraoxacyclotetacosane-tetrone, a migrate from PE packaging (Sage et al., 2018) that is very similar to a NIAS found in biodegradable packaging (Canellas et al., 2015) and tris(2-nonylphenyl) phosphate (in Bio-PE) which is a degradation product of the antioxidant tris(nonylphenyl) phosphite (TNPP) and has been detected in PE (Celiz et al., 2020).

While this creates some confidence in our identification approach, the need to improve databases and workflows cannot be overstated. This is important because (1) we know even less about the chemical composition of starch-, cellulose- and other plant-based materials than bioplastics and (2) manually curating the identification of thousands of features is not feasible. To overcome these challenges, we need to develop community-sourced spectral databases (as in case of environmental pollutants, e.g., NORMAN network) and suspect lists (as for plastic food packaging; Groh et al., 2019).

#### 4.4. Some materials contain more toxic chemicals than others

Based on our results, the chemicals present in the products made of the natural polymers starch and cellulose were toxic on most endpoints. All starch and cellulose products induced baseline toxicity and many contained antiandrogenic compounds. This indicates that the chemicals used in these materials trigger a stronger *in vitro* toxicity than others. Nevertheless, some extracts of PLA and Bio-PE as well as of the materials of which we analyzed only few samples (PHA, Bamboo, Bio-PET, PBS and PBAT) also induced a range of toxicological endpoints, whereas others did not. Here, a generalization, in such that individual materials would induce a specific toxicological signature, is not possible. Instead, the toxicity of these products rather depends on their individual chemical composition. This is supported by our cluster analysis and mirrors our findings on conventional plastics (Zimmermann et al., 2019). Accordingly, we are facing a similar heterogeneity in terms of toxicity in conventional plastics and bio-based/biodegradable materials alike.

On a more positive note, six out of ten Bio-PE products did not

contain toxic chemicals. This implies that bio-based PE formulations are available on the market not containing the substances that induced *in vitro* toxicity. Again, this corresponds to our previous findings on products made of conventional PE (Zimmermann et al., 2019). Here, half of the products were nontoxic in the same bioassays. This is plausible because changing the carbon source of the monomers will only minimally change the chemical composition of the polymer. While some impurities may be different, the reaction by-products and additives will remain the same. Accordingly, shifting from petroleum- to plant-based monomers will probably not affect the toxicity present in the finished material. Such considerations may, however, not apply to Bio-PET. While we did not detect any relevant toxicity in conventional PET (Zimmermann et al., 2019), one of the two Bio-PET samples induced baseline toxicity, triggered an oxidative stress response and was anti-androgenic. Whether this is caused by chemicals specifically used in bio-based PET formulations remains to be investigated.

#### 4.5. Raw materials are less toxic than final products

Across all analyzed endpoints, toxic chemicals were less prevalent and potent in raw materials than in final products. Due to a lack of product information, we do not know whether the analyzed raw materials correspond to the final products. Still, our results indicate that during the conversion of the raw material to the finished product (compounding) new substances are added or generated. This hypothesis is supported by the number of chemical features we observed. Here, we detected overall fewer chemical features in raw materials than in final products of the same material (Tab. 1). As an example, Bio-PE pellets 4 and 8 contained 819 and 186 chemical features, respectively, whereas all but one analyzed Bio-PE product contained more than 900 features. In contrast, the extrusion of bioplastic pellets to a film did not generate new compounds (Aznar et al., 2019). Here, studies analyzing the toxicity of the same raw material and the corresponding finished products can help clarify this question.

#### 4.6. Bioplastics and plant-based materials are not safer than conventional plastics

In our previous work, we analyzed mainly petroleum-based plastics and found toxicity in 67% of the conventional plastics (Zimmermann et al., 2019). Since their bio-based and/or biodegradable counterparts are promoted as sustainable alternatives, we were interested in whether they are indeed safer from a chemical perspective, that is whether they contain less toxic chemicals. Just as for conventional plastics, we detected *in vitro* toxicity in 67% of the bio-based/biodegradable samples using the same bioassays. There were even more bioplastics and plant-based materials than conventional products that triggered baseline toxicity. Regarding effect levels, we detected no significant differences for all toxicological endpoints except for estrogenicity which was less pronounced in bio-based/biodegradable products. To the best of our knowledge, there is no other study that compares the *in vitro* toxicity of conventional plastics and the bio-based/biodegradable alternatives. However, reports on the phytotoxicity indicate that both, starch-based and HDPE bags, released compounds that impaired seedling growth and plant interactions (Balestri et al., 2019; Menicagli et al., 2019). Thus, in this scenario, the chemicals present in natural and synthetic polymers induced a comparable chemical toxicity.

Importantly, the performance and sustainability of bioplastics and plant-based materials cannot be evaluated based on toxicity alone. Here, other environmental (e.g., land and pesticide use, greenhouse gas emissions) and societal impacts (e.g., competition with food production) also need to be taken into account. As life cycle assessments and similar frameworks tend to focus on the latter aspects, an evaluation of the environmental performance and safety of new materials needs to expand to the release of chemicals and particles (e.g., nanoplastics) as well (Ernststoff et al., 2019; Muncke et al., 2020). Only when taking such

holistic view can we “design out” negative properties without getting caught in a loop of regrettable substitutions.

## 5. Conclusions

In this study, we combined *in vitro* bioassays with high resolution non-target mass spectrometry to characterize the toxicity and chemical composition of bio-based and biodegradable materials. Our results indicate that the majority (67%) of bioplastics and plant-based products contain toxic chemicals as well as a large number and diversity of compounds (> 1000 chemical features each in 80% of the samples). Importantly, we applied solvent extraction in order to analyze the intrinsic chemical toxicity present in the products. In future work, migration studies with food simulants are needed in order to identify the toxicity and chemicals migrating under real-world conditions and to estimate the human exposure to those.

Our study demonstrates that bio-based and/or biodegradable materials available on the market are just as toxic as conventional plastics with regards to the chemicals they contain. This highlights that the positive connotation of “biological” or “sustainable” materials does not extend to chemical hazards. Accordingly, our findings imply that in order to develop bio-based/biodegradable materials that indeed outperform conventional plastics, sustainability and chemical safety aspects must be addressed alike. One way to promote this is to integrate chemical toxicity into the life cycle assessment of materials.

On a positive note, we show that safer products are already at the market that can be used as best practice examples. Additionally, the chemical safety of materials can be further optimized using green chemistry to “design out” toxicity during the development of new bio-based and biodegradable materials. Besides these human health aspects, the carbon, energy, water and land footprints need to be minimized to create truly better plastics or plastic alternatives and avoid regrettable substitutions.

## CRedit authorship contribution statement

**Lisa Zimmermann:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Andrea Dombrowski:** Investigation, Writing - review & editing. **Carolin Völker:** Writing - review & editing, Supervision, Funding acquisition. **Martin Wagner:** Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

## 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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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106066>.

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