Adipogenic activity of chemicals used in plastic consumer products

Johannes Völker[1]*, Felicity Ashcroft[1], Åsa Vedøy[1], Lisa Zimmermann[2] and Martin Wagner[1]

[1] Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway

[2] Department of Aquatic Ecotoxicology, Goethe University Frankfurt am Main, 60438 Frankfurt am Main, Germany

*Johannes Völker

Email: johannes.voelker@ntnu.no

This PDF file includes:

Main Text

Figures 1 to 3

Table 1

Abstract

Bisphenols and phthalates, chemicals frequently used in plastic products, promote obesity in cell and animal models. However, these well-known metabolism disrupting chemicals (MDCs) represent only a minute fraction of all compounds found in plastics. To gain a comprehensive understanding of plastics as a source of exposure to MDCs, we characterized all chemicals present in 34 everyday products using nontarget high-resolution mass spectrometry and analyzed their joint adipogenic activities by high-content imaging. We detected 55,300 chemical features and tentatively identified 629 unique compounds, including 11 known MDCs. Importantly, chemicals that induced proliferation, growth, and triglyceride accumulation in 3T3-L1 adipocytes were found in one third of the products. Since the majority did not target peroxisome proliferator-activated receptor γ, the effects are likely to be caused by unknown MDCs. Our study demonstrates that daily-use plastics contain potent mixtures of MDCs and can, therefore, be a relevant yet underestimated environmental factor contributing to obesity.

Teaser

Plastics contain a potent mixture of chemicals promoting adipogenesis, a key process in developing obesity.

Introduction

Obesity is a global pandemic that generates a considerable burden of disease, in particular considering comorbidities such as type 2 diabetes, cardiovascular diseases, hypertension, non-alcoholic fatty liver, stroke, and certain types of cancer (*1*). Worldwide, the number of obese people has nearly tripled since 1975, and more than 41 million children under the age of five were overweight or obese in 2016 (*2*). This is problematic since a high body mass index (BMI) is one of the top risk factors for deaths (*3*), and overweight in childhood or adolescence is a good predictor of adult obesity (*4*). Accordingly, a high BMI contributed to four million deaths globally in 2015 with cardiovascular diseases as leading cause of death followed by diabetes, chronic kidney diseases and cancer (*5*).

This public health problem has mainly been attributed to genetic background and changes in lifestyle, such as diet, exercise, sleep deficiency, and aging. However, epidemiological evidence suggests that these factors insufficiently explain the magnitude and speed of the obesity pandemic (*6*). For instance, even when normalizing for caloric intake and exercise, the BMI of US adults increased by 2.3 kg m^{-2} in 2006 compared to 1998 (*6*). Consequently, identifying and understanding other environmental factors than lifestyle is crucial to manage obesity (*7*). Given that the endocrine system controls appetite, satiety, metabolism, and weight, exposure to endocrine disrupting chemicals is one such factor (*8*). In addition to prominent endocrine disruptors, such as the biocide tributyltin and the pesticide dichlorodiphenyltrichloroethane (DDT), plastic chemicals such as bisphenols or phthalates disrupt metabolic functions and promote obesity in cell and animal experiments (*8*). This is further supported by epidemiological studies that have linked weight gain in humans to bisphenol A (BPA) exposure (*9*), while contradicting outcomes have been reported regarding a link to phthalate exposure (*10-12*).

Considering the chemical complexity of plastic consumer products, bisphenols and phthalates represent only the tip of the iceberg. A final article often consists of one or more polymers, multiple intentionally added substances, such as fillers or additives, as well as non-intentionally added

43 substances, for instance residues from the manufacturing (*13*). Based on regulatory inventories,
44 over 4000 substances are associated with plastic food packaging alone (*13*) and, overall, 10,547
45 chemicals are known to be used in plastics (*14*). Moreover, empirical data suggests that plastics
46 contain more chemicals than currently known. Using nontarget chemical analysis, we detected
47 hundreds to thousands of chemicals in plastic consumer products, most of these remaining
48 unknown (*15*). Importantly, the totality of plastic chemicals in a product was toxic *in vitro*, inducing
49 baseline toxicity, oxidative stress, cytotoxicity, and endocrine effects.
50 Building on these results and the fact that bisphenols and phthalates are known metabolism
51 disrupting chemicals (MDCs; *8, 16, 17*), we hypothesized that MDCs are present in plastic
52 consumer products and that metabolic disruption might represent a common but understudied
53 toxicological property of plastic chemicals. To explore this, we used the same plastic consumer
54 products we have extensively characterized previously (*15*), and investigated the extracts'
55 adipogenic activity in differentiation experiments with murine 3T3-L1 cells. Following exposure to
56 MDCs, 3T3-L1 preadipocyte differentiate into adipocytes, accumulate triglycerides until they finally
57 resemble mature white fat cells (*18*). The bioassay targets the induction of adipogenesis at the
58 cellular level and represents a well-established *in vitro* model for metabolic disruption *in vivo* (*19*).
59 We performed optimization experiments and applied high-content fluorescence microscopy
60 combined with an automated image processing to increase the sensitivity and throughput. We also
61 investigated the underlying mechanisms of the adipogenic response and tested whether the
62 extracted plastic chemicals activate the human peroxisome proliferator-activated receptor gamma
63 (PPARγ) or glucocorticoid receptor (GR). We selected PPARγ as a key regulator of adipogenesis
64 (*20*), and included GR because glucocorticoids are important regulators of lipid metabolism (*21*).
65 Accordingly, an excess of agonists for these receptors is associated with obesogenic effects in
66 animal models and humans (e.g., weight gain; *19*). Moreover, we performed nontarget, ultra-high
67 performance liquid chromatography coupled to a quadrupole of flight spectrometer (LC-QTOF-
68 MS/MS) to characterize the chemicals present in plastics and compare these with a list of
69 compounds known to induce adipogenesis.

Results

Adipogenic activity of plastic consumer products. To exclude cytotoxic effects masking the adipogenic response, we used nuclei count data to assess cytotoxicity. Most extracts were not cytotoxic up to the maximum concentration tested (3 mg plastic well^{-1}), except for PP 4, PUR 3 and PUR 4. The latter two were the most cytotoxic samples with the highest noncytotoxic concentration (HNC) of 0.75 mg plastic well^{-1}. The HNC for PP 4 was 1.5 mg plastic well^{-1} (Fig. S1). To assess the induction of adipogenesis by the plastic extracts, we present the numbers of adipocytes and mature adipocytes in the cell populations and the total lipid droplet count per image for the HNC of each sample. Data were compared to both vehicle and rosiglitazone–treated controls (Fig. 1). Further specifications on the endpoints of the automated image processing, dose-response relationships and example images can be found in the supplementary material (Fig. S2–S17).

The extracts of eleven plastic consumer products induced adipogenesis with four samples having an equal or stronger effect than the maximal response of cells exposed to rosiglitazone (PVC 2 and 4, PP 4, PUR 3). Similarly to rosiglitazone (Fig. 1A), the proliferative effects induced by plastic extracts was driven by an increase in the numbers of adipocytes and mature adipocytes, while the number of preadipocytes remained stable (Fig. 1C). Regarding the polymer type, the most potent extracts were PUR and PVC, with seven out of eight samples inducing adipogenesis, whereas for PP, PS and LDPE only specific samples induced adipogenic responses. In contrast, PET, HDPE, and PLA samples were consistently inactive. The same pattern is reflected by the lipid droplet count data (Fig. 1D). Here, however, some additional samples induced a slight increase in lipid droplets (LDPE 1, PS 1, PP 2).

Given the propensity of environmental pollutants to promote unhealthy adipogenesis, we used the single-cell data to explore a size shift towards hypertrophy and increased accumulation of triglycerides in comparison to rosiglitazone (Fig. 2). We here present the results of one out of four experiments due to the large size of the dataset (results of the other experiments can be found in Fig. S18). Exposure to rosiglitazone dose-dependently increased the lipid content of adipocytes

with a median lipid droplet area of 137 pixels cell^{-1} in the lowest concentration to 290 pixels cell^{-1} in the highest concentration (Fig. 2A). The average fluorescence intensity of the lipid droplets remained stable (Fig. 2B), thus the adipocytes increased in size in response to rosiglitazone but triglyceride accumulation within the droplets remained constant. Compared with the maximal response to rosiglitazone, adipocytes exposed to many of the active plastic extracts had a higher lipid content, resulting both from an increase in adipocyte size and from the amount of triglycerides contained within the lipid droplets. The lipid droplet area per adipocyte was greater in nine out of the eleven active samples with a median increase of 21.6–114% (PS 2–LDPE 4). In line with that, the average fluorescence intensity was higher in ten out of the eleven active samples with a median increase of 25.1–60.4% (PS 2–PVC 4). These effects are consistent over all experiments, except for PVC 3 (Fig. S18).

Reporter gene assays. We observed a higher cytotoxicity of the extracts on the U2OS cells compared to the 3T3-L1 cells with five samples being cytotoxic. The most cytotoxic sample was PP 4 with a HNC of 0.19 mg plastic well^{-1}, followed by PS 2, PP 3 and PLA 1 as well as PVC 2 with a HNC of 0.38 and 0.75 mg plastic well^{-1}, respectively (Tab. S1).

None of the samples activated GR (Fig. S19) and five extracts activated PPARγ (Fig. 3A). PLA1 was the most potent sample with a median receptor activity of 34.7%, followed by PS 2 (24.4%), PVC 2 (10.3%), LDPE 2 (8.4%) and PVC 1 (7.3%). Accordingly, the PPARγ activity of plastic chemicals is a poor predictor of their adipogenic activity (Fig. 3B), except for PVC 2 and PS 2 which induced both PPARγ and adipogenesis at similar effect concentrations. Moreover, three out of the five samples activating PPARγ did not induce adipogenesis in 3T3-L1 cells (PLA 1, LDPE 2, PVC 1).

Chemicals tentatively identified in plastics. Using nontarget GC-QTOF-MS/MS, we previously identified 260 unique chemicals in extracts of the same plastic products (*15*). This corresponds to 231 tentatively identified chemicals with 227 unique PubChem CIDs in the samples used in this study (Tab. S2). In the nontarget LC-QTOF-MS/MS analysis, we detected in total 55,300 features (i.e., unidentified chemicals) across all samples that were only present in samples or had a >10-

123 fold higher abundance compared to the blanks. Here, the number of features in individual samples
124 ranged from 107 (HDPE 2) to 6242 (PUR 3, Tab. 1). In total, 5500 features had spectral MS/MS
125 data we could use for compound identification, out of which we detected between 30 (PS 4) and
126 2117 features (PUR 3) per sample.

127 For tentatively identifying the plastic chemicals, we used the MS/MS data with the MassBank library
128 (14,788 compounds) and three *in silico* fragmented databases of chemicals potentially used in
129 plastics or (pre-)registered for authorization on the European market (in total 75,510 compounds;
130 *22*). These queries resulted in a successful identification of 2364 features across all samples,
131 corresponding to 629 unique chemicals (SM Excel Tab. S1). Accordingly, between 6 (PLA 2) and
132 33 % (PET 1) of the features in each sample were tentatively identified. For the 25 compounds with
133 the highest identification scores (≥ 50) and abundance in the samples, we confirmed the plausibility
134 of the identification by checking whether the compounds are known to be used in plastics (SM Excel
135 Tab. S2). We found that 14 out of 25 compounds are used in plastics, including five plasticizers
136 (e.g., acetyl tributyl citrate), four flame retardants (e.g., tris(2-butoxyethyl) phosphate, tris(3-
137 methylphenyl) phosphate) and multiple processing aids, such as the lubricant 2-nonyl-N-(2-
138 nonylphenyl) aniline, the hardener 4-methylphthalic anhydride and the slip additive (Z)-docos-13-
139 enamide. We also identified compounds that likely migrated from the packed content into the
140 packaging (two octadecanamides used in cosmetics) and one compound that was implausible (the
141 veterinary drug febarbamate).

142 When cross-referencing the chemicals tentatively identified in plastics and a list of known MDCs
143 inducing adipogenesis in 3T3-L1 cells (Tab. S3), we found eleven compounds known to induce
144 adipogenesis *in vitro*. The MDCs present in our samples include four phthalates and six
145 organophosphates (Tab. 1). Benzyl butyl phthalate (BBP), di-butyl phthalate (DBP) and di(2-
146 ethylhexyl) phthalate (DEHP) were present in PVC 4. Di-iso-nonyl phthalate (DINP) was detected
147 in PVC 3 and 4. Diphenyl phosphate (DPP), 2-ethylhexyl diphenyl phosphate (EHDP) and triphenyl
148 phosphate (TPP) were detected in multiple samples. When using raw abundance as a proxy for
149 concentration, high levels of TPP, DPP and EHDP (the MDCs present in at least three samples)

were detected in two to three active PVC samples (Tab. S4). In contrast, the other active samples contain very low levels of these chemicals (PS 2, PP 4, PUR 2, PUR 3). Interestingly, we did not detect organotin compounds or bisphenols (SM Excel Table S1) despite these are known MDCs and thought of as being common in PVC and other plastics (*16, 17*).

Discussion

Adipogenic activity of plastic consumer products. We hypothesized that plastic products contain MDCs based on the fact that a small set of compounds used in plastics is known to induce adipogenesis *in vitro* and *in vivo* (*8, 16, 17*). However, the current focus on few, individual plastic chemicals neglects the chemical complexity of plastic materials given that we know that thousands of compounds are either used in plastics or non-intentionally present (*13, 14*). Thus, we decided to characterize the adipogenic activity of all compounds extractable from plastic consumer products.

Eleven out of 34 products contain chemicals that induce adipogenesis and are, thus, MDCs *in vitro* (Fig. 1). The chemicals extracted from some plastics are not only quite potent but also trigger effects that are similar to or higher than those induced by the reference compound rosiglitazone (PVC 2 and 4, PP 4). Supramaximal efficacies have previously been reported for single compounds, such as dibutyl phthalate and tert-butyl phenyl diphenyl phosphate (*23*) but only at concentrations ≥10 µM, illustrating the potency of the extracted mixtures.

Products with multiple applications, including two FCMs (PS 2, PP 3) and nine non-FCMs, contain adipogenic chemicals. While chemicals migrating from packaging into food represent an obvious source of human exposure (*24*), compounds released from non-FCMs can also contribute via dermal uptake (e.g., PUR 4 shower slippers) or inhalation. For instance, dust contains chemical mixtures that induce adipogenesis (*23*). Here, we show that plastic flooring (e.g., PVC 4) contains MDCs that may contribute to human exposure if they partition into dust. Given the potency of the extracted mixtures and considering our close and constant contact with plastics, it appears

174 plausible that plastic chemicals contribute to an obesogenic environment and, thus, the obesity
175 pandemic.
176 The chemicals present in PVC and PUR products most consistently induce potent adipogenic
177 responses, while compounds extracted from PET, HDPE, and PLA products were inactive. Apart
178 from the PLA samples, this is in line with our previous findings for other toxicity endpoints (*15*). This
179 suggests that PVC and PUR are more likely to contain MDCs compared to other polymers.
180 However, the chemicals extracted from some PP, PS, and LDPE products also induce
181 adipogenesis (Fig. 1). This supports the idea that caution is needed when trying to generalize the
182 occurrence of toxic chemicals based on polymer type (*15*).
183 Unhealthy or dysfunctional adipocytes are part of the obesity phenotype. They are larger in size,
184 have an impaired glucose uptake and insulin signaling, an elevated inflammatory response and
185 decreased respiration (*25*). While we did not investigate the latter characteristics, adipocytes
186 exposed to plastic chemicals were larger and contained more triglycerides compared to those
187 treated with rosiglitazone (Fig. 2). Since rosiglitazone promotes the development of healthy white
188 adipocytes (*26, 27*), these results suggest that exposure to plastic chemicals could shift adipocytes
189 towards an unhealthy phenotype. Similar trends have been reported for a range of MDCs, including
190 BPA (*28*), organotin compounds (*29, 30*) and DEHP (*31*), which we detected in PVC 4 (Tab. 1).
191 Hence, it will be interesting to investigate whether plastic chemicals also trigger the other hallmarks
192 of unhealthy, dysfunctional adipocytes.

193 **Plastic chemicals and adipogenesis**. Using nontarget high resolution mass spectrometry, we
194 show that plastic products contain hundreds to thousands of extractable chemicals; few of those
195 identifiable using spectral libraries and *in silico* tools. This is in line with our previous research (*15,*
196 *22*) and points towards the presence of unknown chemicals in plastics (e.g., non-intentionally
197 added substances). Accordingly, the relatively low identification performance in our study is a result
198 of the limited coverage of chemical databases. These limitations notwithstanding, we tentatively
199 identified a range of known plastic chemicals providing confidence in the accuracy of the
200 identifications.

Plastic products contain known MDCs, including four phthalates (only in PVC 3 and 4) and six organophosphates (Tab. 1). Biomonitoring data suggests that humans are commonly exposed to some of these compounds (*32-34*). As an example, the phthalates DBP and DEHP as well as the flame retardants TPP and TBEP we found in plastics were recently detected in matched maternal and cord blood samples (*35*). Accordingly, plastic products can be one source of exposure to these MDCs.

Known MDCs may explain the adipogenic response to chemicals extracted from some but not all plastic samples. Most active samples contain at least one MDC with TPP, DPP and EHDP being present in multiple samples. Interestingly, the floor covering (PVC 4) contained ten known MDCs. While the active PVC samples contain high levels of TPP, DPP and EHDP, the abundance of these chemicals was very low in the other active samples (Tab. S4). This suggests that other than the known MDCs contribute to the adipogenesis induced by plastic chemicals.

Underlying mechanisms. PPARγ is a key regulator of adipogenesis (*20*), and many MDCs that induce adipogenesis also activate PPARγ (*17*). Despite the common idea that PPARγ activation is a main mechanism via which anthropogenic chemicals trigger adipogenesis, most of the adipogenic plastic samples in fact do not activate this receptor (Fig. 3). Only in two cases (PVC 2, PS 2) does a high PPARγ activity correspond to a strong induction of lipid droplet formation. Moreover, three samples (PLA 1, LDPE 2, PVC 1) activated PPARγ but were inactive in the adipogenesis assay. Thus, the adipogenic effects of the plastic extracts is not necessarily dependent on direct activation of PPARγ and other mechanisms must be involved.

GR is another important nuclear receptor that participates in adipogenesis, and various MDCs activate GR (*36*). In particular, glucocorticoids are essential in inducing adipocyte differentiation (Fig. S20). However, none of the plastic extracts activated GR rendering this an unlikely mechanism of action in this case.

Elucidating the mechanism by which plastic chemicals induce adipogenesis is complex since we are dealing with two black boxes, namely the complex chemical mixtures present in plastics and the multitude of potential mechanism of actions involved in adipogenic responses in 3T3-L1 cells

(*19*). In addition to PPARγ and GR, (ant)agonists of multiple other nuclear receptors, such as the retinoid x receptor α, estrogen receptor, androgen receptor, liver x receptor, thyroid receptor β, have been demonstrated or are discussed to contribute to an adipogenic response (*37*). In the light of the diversity of compounds we detected in plastics, it appears probable that these act via multiple mechanisms that are in most cases PPARγ and GR independent. Although more work needs to be done to elucidate the underlying mechanisms, our results underline the importance of using integrative methods, such as the adipogenesis assay to identify MDCs triggering cellular responses rather than assessing (anta)agonism at selected nuclear receptors.

Limitations and future directions. To the best of our knowledge, this is the first study investigating the adipogenic activity of chemicals extractable from plastic consumer products. Considering the diversity of plastic products and their chemical composition, the sample set is certainly not representative of all plastic chemicals, humans will be exposed to. While it is challenging to comprehensively characterize the human exposure to plastic chemicals from all types of products, given their ubiquity and diversity, a way forward is to prioritize polymer types that are likely to contain MDCs, such as PVC and PUR.

Given that our aim was to investigate whether MDCs are present in plastic products, we used methanol to extract the samples. This simulates a worst-case scenario. Thus, even though we demonstrated that potent (mixtures of) MDCs are present in consumer products, it remains to be investigated whether these will migrate under more realistic conditions into air, water or food, or can be taken up dermally. Using the same samples as in the present study, we recently demonstrated that a significant number of chemicals and *in vitro* toxicity, such as antiandrogenic compounds, migrate into water (*22*). However, it remains unknown if this is also the case for the present MDCs.

Moreover, we analyzed plastic packaging that contained foodstuff or personal care products because we aimed at investigating final products. Since chemical migration is not a one-way street, we cannot exclude that compounds from the contents migrated into the packaging. The detection of compounds used in cosmetics in its packaging underlines this limitation. Such compounds may

255 contribute to the observed adipogenic activity or PPARγ activation and future research should cover
256 unused final packaging.
257 The nontarget chemical analysis resulted in the tentative identification of several MDCs. However,
258 many compounds remain unidentified and there is some likelihood of false-positive identifications.
259 The challenge of a rather low identification success is well-known for environmental pollutants (*38*)
260 and can be addressed by building more comprehensive spectral databases. Recent efforts to build
261 specific databases for plastic chemicals are promising (*13, 14*) but must be complemented with
262 spectral information and non-intentionally added substances. Moreover, we show that known
263 MDCs only partially – if at all – contribute to the adipogenesis induced by plastic extracts. This
264 points towards the presence of unidentified MDCs in plastics. To identify the compounds that are
265 indeed causative for the observed responses, future research should apply effect-directed analysis.
266 Moreover, our results indicate that plastic chemicals may promote a development towards
267 unhealthy adipocytes. However, more evidence is needed to further support this hypothesis. For
268 instance, one needs to extend the adipogenesis assay to cover later stages of adipocyte
269 development and investigate biomarkers of inflammation and metabolic function (e.g., glucose
270 uptake, insulin sensitivity).
271 Taken together, we demonstrated that plastic consumer products contain potent (mixtures of)
272 MDCs inducing adipogenesis *in vitro* via mechanisms that are mostly not mediated via PPARγ and
273 GR. Accordingly, plastic chemicals may contribute to an obesogenic environment considering our
274 constant contact with a broad range of plastic products. Given that the plastic products containing
275 MDCs also contained compounds triggering other toxicological endpoints (*15*), a shift towards
276 chemically less complex plastics represents a way forward to a non-toxic environment.

Materials and Methods

A list of used chemicals is provided in the supplementary material (Tab. S5).

Sample selection and plastic extraction. We used the same 34 plastic samples (Tab. 1) as in Zimmermann *et al.* (*15*). The samples cover petroleum-based polymers with the highest market share (polypropylene (PP) > low density polyethylene (LDPE) > high density polyethylene (HDPE) > polyvinyl chloride (PVC) > polyurethane (PUR) > polyethylene terephthalate (PET) > polystyrene (PS); *39*), and polylactic acid (PLA) as a bio-based alternative. The samples include 21 products with and 13 products without food contact. Further specifications on the sample selection, collection and polymer identification are described in Zimmermann *et al.* (*15*). We extracted 3 g sample with including three procedure blanks (PB 1-3) with methanol and concentrated the extracts to a final volume of 200 µL using dimethyl sulfoxide as a keeper. To contextualize the bioassay results, we use “plastic equivalents” in such that “1 mg plastic” corresponds to the chemicals extracted from 1 mg of plastic. Accordingly, 1 µl extract corresponds to 15 mg plastic. See supplementary text for details.

Bioassays. We performed differentiation assays with murine 3T3-L1 adipocytes (Zenbio Inc., SP-L1-F, lot 3T3L1062104) to examine the induction of adipogenesis as well as CALUX reporter gene assays (BioDetection Systems B.V., Amsterdam, The Netherlands) to investigate the agonistic activity at the human peroxisome proliferator-activated receptor γ (*40*) and glucocorticoid receptor (GR, *41*). All experiments were conducted with negative controls, vehicle controls, positive controls, and PB 1–3. Samples, controls, and blanks were diluted 1000-fold (adipogenesis assay) or 500-fold (reporter gene assays) with medium, resulting in a maximum final solvent concentration of 0.1% or 0.2% (v/v), respectively. Each sample was analyzed in serial dilutions of 1:2 with four replicates per concentration in at least three independent experiments per assay. Moreover, the respective reference compound was included on every microtiter plate to control for potential variations between plates, and the sample arrangement was randomized to exclude position

effects. As negative and vehicle controls did not differ significantly, the results from both were pooled. Further, there was no contamination during sample extraction and analysis since none of the controls and blanks induced activity (Fig. S21 and S22). See supplementary text for details on the cell culture conditions.

Adipogenesis assay. We performed the differentiation assay with 3T3-L1 cells in accordance with a previously described method (*42*). In brief, an experiment consists of three days pre-differentiation (one day seeding, two days allowing cells to enter the resting state) followed by an 8-d differentiation window (two days differentiation, six days maintenance). Subconfluent cells of passage 10 were trypsinized and counted with a flow cytometer (NovoCyte, Acea Biosciences). 15,000 cells well^{-1} were seeded in 200 µL preadipocyte medium (PAM: DMEM-high supplemented with 10% bovine calf serum and 1% penicillin/streptomycin) into 96-well black, clear bottom tissue culture plates (655090, Greiner Bio-One) and incubated at 37 °C and 5% CO_2. After 24 h, we checked that the cells reached confluency, replaced the medium with 200 µL fresh PAM well^{-1}, and cultured the cells for another 48 h to initiate growth arrest. We included on every plate a pre-adipocyte control (undifferentiated cells) which was kept in PAM, while the rest of the cells were differentiate as described below.

Optimization experiments. Given that a systematic analysis of dexamethasone (DEX) effects on triglyceride accumulation and differentiation efficiency in 3T3-L1 cells was lacking, we conducted optimization experiments to identify a suitable DEX concentration to initiate adipocyte differentiation that results in the lowest baseline as well as the highest sensitivity and dynamic range when co-exposed to the reference compound rosiglitazone. Moreover, we compared two methods to quantify triglycerides based on Nile Red Staining. We determined the total NileRed fluorescence well^{-1} and compared it to an automated imaging and analysis platform to determine whether the latter improves the sensitivity and dynamic range for the screening of adipogenic activity.

Based on the results (Fig. S20) and in comparison with previous studies, we found that a rather low optimal DEX concentration (6.25 nM) was sufficient to initiate adipocyte differentiation without

increasing the assay's baseline. Compared to the fluorescence readout well^{-1}, an automated imaging approach is more sensitive to assess proliferation, enhances the dynamic range of the assay (Fig. S20) and provides more information because it enables single-cell analysis for a more in-depth characterization of the adipocyte population (pre-adipocytes, adipocytes and mature adipocytes). Accordingly, we analyzed the effects of the plastic extracts using 6.25 nM DEX during the differentiation window and the automated imaging approach. See supplementary text for details.

Dosing of samples. To initiate differentiation, we replaced the PAM medium with 200 µL differentiation medium well^{-1} (DM: DMEM-high supplemented with 10% FBS, 1% penicillin/streptomycin, 20 mM HEPES, 1 µg mL^{-1} insulin, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 6.25 nM DEX) containing 5 concentrations of samples serially diluted 1:2 (0.19–3 mg plastic well^{-1} equivalent to 0.94–15 mg plastic mL^{-1}) or rosiglitazone (1.17–300 nM). Following the 48-h differentiation, the medium was replaced with 200 µL adipocyte maintenance medium well^{-1} (DM without IBMX and DEX) containing the respective controls, samples, or rosiglitazone and changed the medium every other day during the 6d maintenance period.

Fixation and staining. After 11 d, cells were fixed with 2% paraformaldehyde and co-stained with NileRed and NucBlue. Imaging was carried out on the Cytation 5 Cell Imaging Multimode reader (BioTek). Three images per field (Brightfield, NucBlue and NileRed) and nine fields per well were captured. See supplementary text for details.

Image analysis. Images were analyzed in the open-source software CellProfiler (*43*). A description of the image analysis protocol and the CellProfiler pipelines are available in the supplementary material. We quantified proliferation (nuclei count), number of lipid droplets (lipid droplet count), total area occupied by lipid droplets (total area), and the total intensity of the NileRed staining within the lipid droplets (total intensity) per image. We further quantified the total area occupied by lipid (lipid droplet area), and the total intensity of NileRed staining in the lipid droplet area per cell.

353 Analysis of the single-cell data was applied to quantify the numbers of adipocytes and mature
354 adipocytes per image. An adipocyte was defined as a cell containing at least one lipid droplet and
355 a mature adipocyte was defined as a cell containing a total lipid content equivalent to ≥ 8 average
356 sized lipid droplets (1000 pixels).

357 Single-cell measurements of lipid droplet area, and NileRed staining were analyzed further to
358 explore how triglyceride accumulation was distributed within an adipocyte population. To control
359 for potential cross-plate differences in staining intensity within an experiment, the average
360 fluorescence intensity per adipocyte was normalized to the mean average fluorescence intensity
361 for an internal plate control (cells treated with 300 nM rosiglitazone). For an independent
362 experiment, single-cell data were grouped based on the treatment. The median lipid droplet area
363 and normalized average fluorescence intensities per cell were calculated for each experiment. An
364 example of the images and visualized output of the image analysis is shown in Fig. S2.

365 **Reporter gene assays**. We performed the CALUX reporter gene assays in 384-well plates and
366 used imaging to count nuclei per well to normalize the reporter gene response and assess
367 cytotoxicity. Rosiglitazone was the reference compound for PPARγ and DEX for GR (Fig. S23).
368 See supplementary text for the detailed protocol.

369 **Analysis of bioassay data**. We used GraphPad Prism 9 (GraphPad Software, San Diego, CA) for
370 non-linear regressions and statistical analysis, and interpolated plastic equivalents inducing 10 or
371 20% effect (effect concentration, EC_{10} or EC_{20}) from the respective dose-response curves. The limit
372 of detection (LOD) of each endpoint and experiment was calculated as three times the standard
373 deviation (SD) of pooled controls. See supplementary text for details.

374 **Nontarget chemical analysis**. We analyzed all samples, except PLA 3, using ultra-high
375 performance liquid chromatography coupled to a quadrupole time of flight spectrometer (LC-QTOF-
376 MS/MS) with an Acquity UPLC Waters liquid chromatography system coupled to a SYNAPT G2-S
377 mass spectrometer (both Waters Norge, Oslo, Norway). The analytical method has been described

in Zimmermann *et al.* (*22, 44*) and a brief description as well as information about the data analysis and compound identification can be found in the supplementary text.

Comparison with chemicals known to induce adipogenesis. We built a list of 120 know adipogenic chemicals (Tab. S3) by searching Web of Science (Core Collection) for studies investigating chemicals in the adipogenesis assay and complemented the search with chemicals reviewed by Amato *et al.* (*16*). We cross-referenced the list with the tentatively identified compounds in the plastic samples to determine whether some of these compounds are MDCs (Tab. 1). See supplementary text for details.

References

1. B. A. Swinburn, V. I. Kraak, S. Allender, V. J. Atkins, P. I. Baker, J. R. Bogard, *et al.*, The global syndemic of obesity, undernutrition, and climate change: The Lancet Commission report. Lancet 393, 791-846 (2019).

2. WHO, Obesity and overweight. Fact sheet, World Health Organization, Geneva, Switzerland. [cited 2021 August 24] Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

3. E. Gakidou, A. Afshin, A.A. Abajobir, K.H. Abate, C. Abbafati, K.M. Abbas, *et al.*, Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390, 1345-1422 (2017).

4. M. Simmonds, A. Llewellyn, C. G. Owen, N. Woolacott, Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev 17, 95-107 (2016).

5. A. Afshin, M. H. Forouzanfar, M. B. Reitsma, P. Sur, K. Estep, A. Lee, *et al.*, Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med 377, 13-27 (2017).

6. R. E. Brown, A. M. Sharma, C. I. Ardern, P. Mirdamadi, P. Mirdamadi, J. L. Kuk, Secular differences in the association between caloric intake, macronutrient intake, and physical activity with obesity. Obes Res Clin Pract 10, 243-255 (2016).

7. P. W. Franks, M. I. McCarthy, Exposing the exposures responsible for type 2 diabetes and obesity. Science 354, 69-73 (2016).

8. J. J. Heindel, B. Blumberg, M. Cave, R. Machtinger, A. Mantovani, M. A. Mendez, A. Nadal, P. Palanza, G. Panzica, R. Sargis, L. N. Vandenberg, F. Vom Saal, Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol 68, 3-33 (2017).

9. L. A. Hoepner, R. M. Whyatt, E. M. Widen, A. Hassoun, S. E. Oberfield, N. T. Mueller, D. Diaz, A. M. Calafat, F. P. Perera, A. G. Rundle, Bisphenol A and adiposity in an inner-city birth cohort. Environ Health Perspect 124, 1644-1650 (2016).

10. L. Trasande, T. M. Attina, S. Sathyanarayana, A. J. Spanier, J. Blustein, Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect 121, 501-506 (2013).

11. D. Valvi, M. Casas, D. Romaguera, N. Monfort, R. Ventura, D. Martinez, J. Sunyer, M. Vrijheid, Prenatal phthalate exposure and childhood growth and blood pressure: Evidence from the Spanish INMA-Sabadell birth cohort atudy. Environ Health Perspect 123, 1022-1029 (2015).

12. J. P. Buckley, S. M. Engel, M. A. Mendez, D. B. Richardson, J. L. Daniels, A. M. Calafat, M. S. Wolff, A. H. Herring, Prenatal phthalate exposures and childhood fat mass in a New York city cohort. Environ Health Perspect 124, 507-513 (2016).

13. K. J. Groh, T. Backhaus, B. Carney-Almroth, B. Geueke, P. A. Inostroza, A. Lennquist, H. A. Leslie, M. Maffini, D. Slunge, L. Trasande, A. M. Warhurst, J. Muncke, Overview of known plastic packaging-associated chemicals and their hazards. Sci Total Environ 651, 3253-3268 (2019).

14. H. Wiesinger, Z. Wang, S. Hellweg, Deep dive into plastic monomers, additives, and processing aids. Environ Sci Technol 55, 9339-9351 (2021).

15. L. Zimmermann, G. Dierkes, T. A. Ternes, C. Völker, M. Wagner, Benchmarking the in vitro toxicity and chemical composition of plastic consumer products. Environ Sci Technol 53, 11467-11477 (2019).

16. A. A. Amato, H. B. Wheeler, B. Blumberg, Obesity and endocrine-disrupting chemicals. Endocr Connect 10, R87-R105 (2021).

17. R. J. Egusquiza, B. Blumberg, Environmental obesogens and their impact on susceptibility to obesity: New mechanisms and chemicals. Endocrinology 161, 1-14 (2020).

18. H. Green, M. Meuth, An established pre-adipose cell line and its differentiation in culture. Cell 3, 127-133 (1974).

19. C. D. Kassotis, H. M. Stapleton, Endocrine-mediated mechanisms of metabolic disruption and new approaches to examine the public health threat. Front Endocrinol 10, 39 (2019).

20. E. D. Rosen, O. A. MacDougald, Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7, 885-896 (2006).

21. K. John, J. S. Marino, E. R. Sanchez, T. D. Hinds, Jr., The glucocorticoid receptor: cause of or cure for obesity? Am J Physiol Endocrinol Metab 310, E249-257 (2016).

22. L. Zimmermann, Z. Bartosova, K. Braun, J. Oehlmann, C. Völker, M. Wagner, Plastic Products Leach Chemicals That Induce In Vitro Toxicity under Realistic Use Conditions. Environ Sci Technol, (2021). Available at: https://doi.org/10.1021/acs.est.1c01103

23. C. D. Kassotis, K. Hoffman, H. M. Stapleton, Characterization of adipogenic activity of house dust extracts and semi-volatile indoor contaminants in 3T3-L1 Cells. Environ Sci Technol 51, 8735-8745 (2017).

24. J. Muncke, T. Backhaus, B. Geueke, M. V. Maffini, O. V. Martin, J. P. Myers, A. M. Soto, L. Trasande, X. Trier, M. Scheringer, Scientific challenges in the risk assessment of food contact materials. Environ Health Perspect 125, 095001 (2017).

25. A. L. Ghaben, P. E. Scherer, Adipogenesis and metabolic health. Nat Rev Mol Cell Biol 20, 242-258 (2019).

26. A. M. Sharma, B. Staels, Review: Peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab 92, 386-395 (2007).

27. C. M. Kusminski, P. E. Bickel, P. E. Scherer, Targeting adipose tissue in the treatment of obesity-associated diabetes. Nat Rev Drug Discov 15, 639-660 (2016).

28. F. Ariemma, V. D'Esposito, D. Liguoro, F. Oriente, S. Cabaro, A. Liotti, I. Cimmino, M. Longo, F. Beguinot, P. Formisano, R. Valentino, Low-Dose bisphenol-A impairs adipogenesis and generates dysfunctional 3T3-L1 adipocytes. Plos One 11, e0150762 (2016).

29. S. M. Regnier, E. El-Hashani, W. Kamau, X. Zhang, N. L. Massad, R. M. Sargis, Tributyltin differentially promotes development of a phenotypically distinct adipocyte. Obesity 23, 1864-1871 (2015).

30. B. M. Shoucri, V. T. Hung, R. Chamorro-Garcia, T. Shioda, B. Blumberg, Retinoid x receptor activation during adipogenesis of female mesenchymal stem cells programs a dysfunctional adipocyte. Endocrinology 159, 2863-2883 (2018).

31. N. Kloting, N. Hesselbarth, M. Gericke, A. Kunath, R. Biemann, R. Chakaroun, J. Kosacka, P. Kovacs, M. Kern, M. Stumvoll, B. Fischer, U. Rolle-Kampczyk, R. Feltens, W. Otto, D. K. Wissenbach, M. von Bergen, M. Bluher, Di-(2-ethylhexyl)-phthalate (DEHP) causes impaired adipocyte function and alters serum metabolites. Plos One 10, e0143190 (2015).

32. M. Ospina, N. K. Jayatilaka, L. Y. Wong, P. Restrepo, A. M. Calafat, Exposure to organophosphate flame retardant chemicals in the U.S. general population: Data from the 2013-2014 National Health and Nutrition Examination Survey. Environ Int 110, 32-41 (2018).

475 33. A. R. Zota, C. A. Phillips, S. D. Mitro, Recent fast food consumption and bisphenol A and
476 phthalates exposures among the U.S. population in NHANES, 2003-2010. Environ Health
477 Perspect 124, 1521-1528 (2016).

478 34. A. R. Zota, A. M. Calafat, T. J. Woodruff, Temporal trends in phthalate exposures:
479 Findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ Health
480 Perspect 122, 235-241 (2014).

481 35. A. Wang, D. P. Abrahamsson, T. Jiang, M. Wang, R. Morello-Frosch, J. S. Park, M.
482 Sirota, T. J. Woodruff, Suspect screening, prioritization, and confirmation of environmental
483 chemicals in maternal-newborn pairs from San Francisco. Environ Sci Technol 55, 5037-5049
484 (2021).

485 36. R. M. Sargis, D. N. Johnson, R. A. Choudhury, M. J. Brady, Environmental endocrine
486 disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation.
487 Obesity 18, 1283-1288 (2010).

488 37. M. Fu, T. Sun, A. L. Bookout, M. Downes, R. T. Yu, R. M. Evans, D. J. Mangelsdorf, A
489 nuclear receptor atlas: 3T3-L1 adipogenesis. Mol Endocrinol 19, 2437-2450 (2005).

490 38. J. Hollender, E. L. Schymanski, H. P. Singer, P. L. Ferguson, Nontarget screening with
491 high resolution mass spectrometry in the environment: Ready to go? Environ Sci Technol 51,
492 11505-11512 (2017).

493 39. PlasticEurope, Plastics-The Facts 2020: An analysis of european plastics production,
494 demand and waste data (2020). [cited 2021 August 24] Available at:
495 https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020

496 40. L. Gijsbers, H. Y. Man, S. K. Kloet, L. H. de Haan, J. Keijer, I. M. Rietjens, B. van der
497 Burg, J. M. Aarts, Stable reporter cell lines for peroxisome proliferator-activated receptor gamma
498 (PPARgamma)-mediated modulation of gene expression. Anal Biochem 414, 77-83 (2011).

499 41. E. Sonneveld, H. J. Jansen, J. A. Riteco, A. Brouwer, B. van der Burg, Development of
500 androgen- and estrogen-responsive bioassays, members of a panel of human cell line-based
501 highly selective steroid-responsive bioassays. Toxicol Sci 83, 136-148 (2005).

502 42. C. D. Kassotis, L. Masse, S. Kim, J. J. Schlezinger, T. F. Webster, H. M. Stapleton,
503 Characterization of adipogenic chemicals in three different cell culture systems: Implications for
504 reproducibility based on cell source and handling. Sci Rep 7, 42104 (2017).

505 43. A. E. Carpenter, T. R. Jones, M. R. Lamprecht, C. Clarke, I. H. Kang, O. Friman, D. A.
506 Guertin, J. H. Chang, R. A. Lindquist, J. Moffat, P. Golland, D. M. Sabatini, CellProfiler: Image
507 analysis software for identifying and quantifying cell phenotypes. Genome Biol 7, R100 (2006).

508 44. L. Zimmermann, A. Dombrowski, C. Völker, M. Wagner, Are bioplastics and plant-based
509 materials safer than conventional plastics? In vitro toxicity and chemical composition. Environ Int
510 145, 106066 (2020).

511 45. K. Zebisch, V. Voigt, M. Wabitsch, M. Brandsch, Protocol for effective differentiation of
512 3T3-L1 cells to adipocytes. Anal Biochem 425, 88-90 (2012).

513 46. V. A. Chappell, A. Janesick, B. Blumberg, S. E. Fenton, Tetrabromobisphenol-A
514 promotes early adipogenesis and lipogenesis in 3T3-L1 Cells. Toxicol Sci 166, 332-344 (2018).

515 47. J. G. Boucher, A. Boudreau, S. Ahmed, E. Atlas, In vitro effects of bisphenol A beta-D-
516 glucuronide (BPA-G) on adipogenesis in human and murine preadipocytes. Environ Health
517 Perspect 123, 1287-1293 (2015).

518 48. V. Pomatto, E. Cottone, P. Cocci, M. Mozzicafreddo, G. Mosconi, E. R. Nelson, F. A.
519 Palermo, P. Bovolin, Plasticizers used in food-contact materials affect adipogenesis in 3T3-L1
520 cells. J Steroid Biochem Mol Biol 178, 322-332 (2018).

49. A. Riu, M. Grimaldi, A. le Maire, G. Bey, K. Phillips, A. Boulahtouf, E. Perdu, D. Zalko, W. Bourguet, P. Balaguer, Peroxisome proliferator-activated receptor gamma is a target for halogenated analogs of bisphenol A. Environ Health Persp 119, 1227-1232 (2011).

50. V. Adomshick, Y. Pu, A. Veiga-Lopez, Automated lipid droplet quantification system for phenotypic analysis of adipocytes using CellProfiler. Toxicol Mech Methods 30, 378-387 (2020).

51. E. L. Schymanski, H. P. Singer, J. Slobodnik, I. M. Ipolyi, P. Oswald, M. Krauss, T. Schulze, P. Haglund, T. Letzel, S. Grosse, N. S. Thomaidis, A. Bletsou, C. Zwiener, M. Ibanez, T. Portoles, R. de Boer, M. J. Reid, M. Onghena, U. Kunkel, W. Schulz, A. Guillon, N. Noyon, G. Leroy, P. Bados, S. Bogialli, D. Stipanicev, P. Rostkowski, J. Hollender, Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal Bioanal Chem 407, 6237-6255 (2015).

52. W. J. Sun, X. Y. Duan, H. Chen, L. Y. Zhang, H. W. Sun, Adipogenic activity of 2-ethylhexyl diphenyl phosphate via peroxisome proliferator-activated receptor gamma pathway. Sci Total Environ 711, 134810 (2020).

53. J. Blanco, L. Guardia-Escote, M. Mulero, P. Basaure, J. Biosca-Brull, M. Cabre, M. T. Colomina, J. L. Domingo, D. J. Sanchez, Obesogenic effects of chlorpyrifos and its metabolites during the differentiation of 3T3-L1 preadipocytes. Food Chem Toxicol 137, 111171 (2020).

54. Z. D. Sun, H. M. Cao, Q. S. Liu, Y. Liang, H. Fiedler, J. Q. Zhang, Q. F. Zhou, G. B. Jiang, 4-Hexylphenol influences adipogenic differentiation and hepatic lipid accumulation in vitro. Environ Pollut 268, 115365 (2021).

55. C. D. Kassotis, E. M. Kollitz, P. L. Ferguson, H. M. Stapleton, Nonionic ethoxylated surfactants induce adipogenesis in 3T3-L1 Cells. Toxicol Sci 162, 124-136 (2018).

56. C. J. Hao, X. J. Cheng, H. F. Xia, X. Ma, The endocrine disruptor 4-nonylphenol promotes adipocyte differentiation and induces obesity in mice. Cell Physiol Biochem 30, 382-394 (2012).

57. A. S. Janesick, G. Dimastrogiovanni, L. Vanek, C. Boulos, R. Chamorro-Garcia, W. Tang, B. Blumberg, On the utility of ToxCast and ToxPi as methods for identifying new obesogens. Environ Health Perspect 124, 1214-1226 (2016).

58. F. V. Andrews, S. M. Kim, L. Edwards, J. J. Schlezinger, Identifying adipogenic chemicals: Disparate effects in 3T3-L1, OP9 and primary mesenchymal multipotent cell models. Toxicol in Vitro 67, 1634-1643 (2020).

59. L. B. Sales, J. H. Kamstra, P. H. Cenijn, L. S. Rijt, T. Hamers, J. Legler, Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicol in Vitro 27, 1634-1643 (2013).

60. L. Yin, K. S. Yu, K. Lu, X. Z. Yu, Benzyl butyl phthalate promotes adipogenesis in 3T3-L1 preadipocytes: A high content cellomics and metabolomic analysis. Toxicol in Vitro 32, 297-309 (2016).

61. Y. F. Wang, H. R. Chao, C. H. Wu, C. H. Tseng, Y. T. Kuo, T. C. Tsou, A recombinant peroxisome proliferator response element-driven luciferase assay for evaluation of potential environmental obesogens. Biotechnol Lett 32, 1789-1796 (2010).

62. A. Pereira-Fernandes, H. Demaegdt, K. Vandermeiren, T. L. M. Hectors, P. G. Jorens, R. Blust, C. Vanparys, Evaluation of a screening system for obesogenic compounds: Screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. Plos One 8, e77481 (2013).

63. C. S. We, X. T. Wang, X. P. Yao, F. X. Xi, Y. L. He, Y. T. Xu, L. Ma, X. C. Chen, C. Zhao, R. R. Du, W. J. Pang, G. S. Yang, T. Y. Yu, Bifenthrin induces fat deposition by improving fatty acid uptake and inhibiting lipolysis in mice. J Agric and Food Chem 67, 14048-14055 (2019).

64. M. A. Martinez, J. Blanco, J. Rovira, V. Kumara, J. L. Domingo, M. Schuhmacher, Bisphenol A analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line. Food Chem Toxicol 140, 111298 (2020).

65. S. I. Choi, J. S. Lee, S. R. Lee, W. S. Sim, Y. C. Kim, O. H. Lee, Potentilla rugulosa nakai extract attenuates bisphenol A-, S- and F-induced ROS production and differentiation of 3T3-L1 preadipocytes in the absence of dexamethasone. Antioxidants 9, 113 (2020).

66. Z. Drobna, A. Talarovicova, H. E. Schrader, T. R. Fennell, R. W. Snyder, E. F. Rissman, Bisphenol F has different effects on preadipocytes differentiation and weight gain in adult mice as compared with bisphenol A and S. Toxicology 420, 66-72 (2019).

67. R. Chamorro-Garcia, S. Kirchner, X. Li, A. Janesick, S. C. Casey, C. Chow, B. Blumberg, Bisphenol A diglycidyl ether induces adipogenic differentiation of multipotent stromal stem cells through a peroxisome proliferator-activated receptor gamma-independent mechanism. Environ Health Persp 120, 984-989 (2012).

68. C. Helies-Toussaint, L. Peyre, C. Costanzo, M. C. Chagnon, R. Rahmani, Is bisphenol S a safe substitute for bisphenol A in terms of metabolic function? An in vitro study. Toxicol Appl Pharm 280, 224-235 (2014).

69. J. M. Hall, H. A. Powell, L. Rajic, K. S. Korach, The role of dietary phytoestrogens and the nuclear receptor PPAR gamma in adipogenesis: An in vitro study. Environ Health Persp 127, 37007 (2019).

70. V. Peshdary, G. Calzadilla, A. Landry, A. Sorisky, E. Atlas, Dechlorane Plus increases adipogenesis in 3T3-L1 and human primary preadipocytes independent of peroxisome proliferator-activated receptor gamma transcriptional activity. Int J Obes 43, 545-555 (2019).

71. J. N. Feige, L. Gelman, D. Rossi, V. Zoete, R. Metivier, C. Tudor, S. I. Anghel, A. Grosdidier, C. Lathion, Y. Engelborghs, O. Michielin, W. Wahli, B. Desvergne, The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis. J Biol Chem 282, 19152-19166 (2007).

72. M. Biserni, R. Mesnage, R. Ferro, E. Wozniak, T. Xenakis, C. A. Mein, M. N. Antoniou, Quizalofop-p-ethyl induces adipogenesis in 3T3-L1 Adipocytes. Toxicol Sci 170, 452-461 (2019).

73. R. Mesnage, M. Biserni, D. Genkova, L. Wesolowski, M. N. Antoniou, Evaluation of neonicotinoid insecticides for oestrogenic, thyroidogenic and adipogenic activity reveals imidacloprid causes lipid accumulation. J Appl Toxicol 38, 1483-1491 (2018).

74. A. Smith, X. Z. Yu, L. Yin, Diazinon exposure activated transcriptional factors CCAAT-enhancer-binding proteins alpha (C/EBP alpha) and peroxisome proliferator-activated receptor gamma (PPAR gamma) and induced adipogenesis in 3T3-L1 preadipocytes. Pestic Biochem Physiol 150, 48-58 (2018).

75. A. M. Temkin, R. R. Bowers, M. E. Magaletta, S. Holshouser, A. Maggi, P. Ciana, L. J. Guillette, J. A. Bowden, J. R. Kucklick, J. E. Baatz, D. D. Spyropoulos, Effects of crude oil/dispersant mixture and dispersant components on PPAR gamma activity in vitro and in vivo: Identification of dioctyl sodium sulfosuccinate (DOSS; CAS #577-11-7) as a probable obesogen. Environ Health Perspect 124, 112-119 (2016).

76. R. R. Bowers, A. M. Temkin, L. J. Guillette, J. E. Baatz, D. D. Spyropoulos, The commonly used nonionic surfactant Span 80 has RXR alpha transactivation activity, which likely increases the obesogenic potential of oil dispersants and food emulsifiers. Gen Comp Endocrinol 238, 61-68 (2016).

77. G. Cano-Sancho, A. Smith, M. A. La Merrill, Triphenyl phosphate enhances adipogenic differentiation, glucose uptake and lipolysis via endocrine and noradrenergic mechanisms. Toxicol in Vitro 40, 280-288 (2017).

615 78. X. N. Xie, C. X. Yu, Q. D. Ren, Q. Wen, C. X. Zhao, Y. Tang, Y. G. Du, Exposure to
616 HBCD promotes adipogenesis both in vitro and in vivo by interfering with Wnt6 expression. Sci
617 Total Environ 705, 67-77 (2020).

618 79. E. W. Y. Tung, S. Ahmed, V. Peshdary, E. Atlas, Firemaster (R) 550 and its components
619 isopropylated triphenyl phosphate and triphenyl phosphate enhance adipogenesis and
620 transcriptional activity of peroxisome proliferator activated receptor (Ppar gamma) on the
621 adipocyte protein 2 (aP2) promoter. Plos One 12, e0175855 (2017).

622 80. S. E. Elmore, G. Cano-Sancho, M. A. La Merrill, Disruption of normal adipocyte
623 development and function by methyl- and propyl- paraben exposure. Toxicol Lett 334, 27-35
624 (2020).

625 81. C. J. Hao, X. J. Cheng, H. F. Xia, X. Ma, The endocrine disruptor mono-(2-ethylhexyl)
626 phthalate promotes adipocyte differentiation and induces obesity in mice. Biosci Repo 32, 619-
627 629 (2012).

628 82. Q. Wen, X. N. Xie, C. F. Zhao, Q. D. Ren, X. Y. Zhang, D. B. Wei, B. Emanuelli, Y. G.
629 Du, The brominated flame retardant PBDE 99 promotes adipogenesis via regulating mitotic clonal
630 expansion and PPAR gamma expression. Sci Total Environ 670, 67-77 (2019).

631 83. A. M. Watkins, C. R. Wood, M. T. Lin, B. D. Abbott, The effects of perfluorinated
632 chemicals on adipocyte differentiation in vitro. Mol Cell Endocrinol 400, 90-101 (2015).

633 84. Y. Ma, J. Yang, Y. J. Wan, Y. Peng, S. Ding, Y. Y. Li, B. Xu, X. Chen, W. Xia, Y. B. Ke, S.
634 Q. Xu, Low-level perfluorooctanoic acid enhances 3T3-L1 preadipocyte differentiation via altering
635 peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation.
636 J Appl Toxicol 38, 398-407 (2018).

637 85. V. A. Chappell, A. Janesick, B. Blumberg, S. E. Fenton, Tetrabromobisphenol-A
638 promotes early adipogenesis and lipogenesis in 3T3-L1 Cells. Toxicol Sci 166, 332-344 (2018).

639 86. B. A. Neel, M. J. Brady, R. M. Sargis, The endocrine disrupting chemical tolylfluanid
640 alters adipocyte metabolism via glucocorticoid receptor activation. Mol Endocrinol 27, 394-406
641 (2013).

642 87. A. Pereira-Fernandes, C. Vanparys, T. L. M. Hectors, L. Vergauwen, D. Knapen, P. G.
643 Jorens, R. Blust, Unraveling the mode of action of an obesogen: Mechanistic analysis of the
644 model obesogen tributyltin in the 3T3-L1 cell line. Mol Cell Endocrinol 370, 52-64 (2013).

645 88. F. Grun, H. Watanabe, Z. Zamanian, L. Maeda, K. Arima, R. Chubacha, D. M. Gardiner,
646 J. Kanno, T. Iguchi, B. Blumberg, Endocrine-disrupting organotin compounds are potent inducers
647 of adipogenesis in vertebrates. Mol Endocrinol 20, 2141-2155 (2006).

648 89. H. Inadera, A. Shimomura, Environmental chemical tributyltin augments adipocyte
649 differentiation. Toxicol Lett 159, 226-234 (2005).

650 90. S. Kim, A. Li, S. Monti, J. J. Schlezinger, Tributyltin induces a transcriptional response
651 without a brite adipocyte signature in adipocyte models. Arch Toxicol 92, 2859-2874 (2018).

652 91. X. Li, J. Ycaza, B. Blumberg, The environmental obesogen tributyltin chloride acts via
653 peroxisome proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1
654 preadipocytes. J Steroid Biochem 127, 9-15 (2011).

655 92. S. M. Regnier, E. El-Hashani, W. Kamau, X. J. Zhang, N. L. Massad, R. M. Sargis,
656 Tributyltin differentially promotes development of a phenotypically distinct adipocyte. Obesity 23,
657 1864-1871 (2015).

658 93. M. Y. Dong, P. H. Yuan, Y. C. Song, H. H. Lei, G. Chen, X. H. Zhu, F. Wu, C. Chen, C. X.
659 Liu, Z. J. Shi, L. M. Zhang, In vitro effects of triclocarban on adipogenesis in murine preadipocyte
660 and human hepatocyte. J Hazard Mater 399, 122829 (2020).

661 94. X. Li, H. T. Pham, A. S. Janesick, B. Blumberg, Triflumizole is an obesogen in mice that
662 acts through peroxisome proliferator activated receptor gamma (PPAR gamma). Environ Health
663 Perspect 120, 1720-1726 (2012).

664 95. S. Kim, N. Rabhi, B. C. Blum, R. Hekman, K. Wynne, A. Emili, S. Farmer, J. J.
665 Schlezinger, Triphenyl phosphate is a selective PPAR gamma modulator that does not induce
666 brite adipogenesis in vitro and in vivo. Arch Toxicol 94, 3087-3103 (2020).

667

Acknowledgments

This study was support by internal funding of the Norwegian University of Science and Technology (NTNU, Trondheim). We thank Susana Villa Gonzales (NTNU) for UPLC-QTOF-MS/MS training and support.

Author contributions

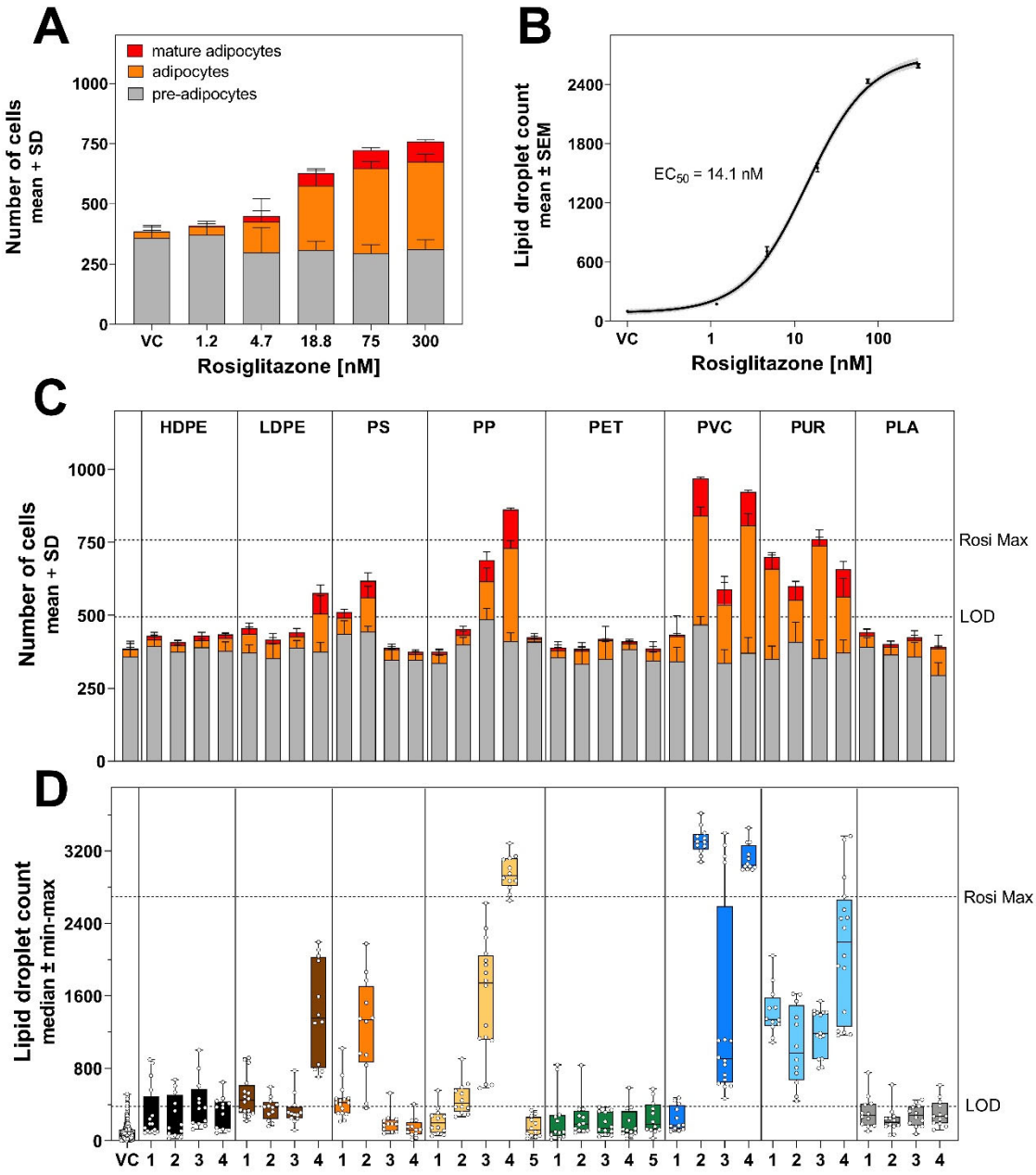
J.V., F.A. and M.W. conceived the study, J.V. and Å.V. performed the experiments, L.Z. performed the chemical analysis, J.V., F.A., and M.W. analyzed the data and wrote the manuscript, and all authors provided comments on the manuscript.

Competing interest

LZ became an employee the Food Packaging Forum (FPF) after this study was concluded. MW is an unremunerated member of the Scientific Advisory Board of the FPF and received travel support for attending annual SAB meetings. FPF is a Swiss foundation that enhances the scientific principles and recent scientific findings that are relevant to the topic of food contact chemicals and their health impacts on humans and the environment.

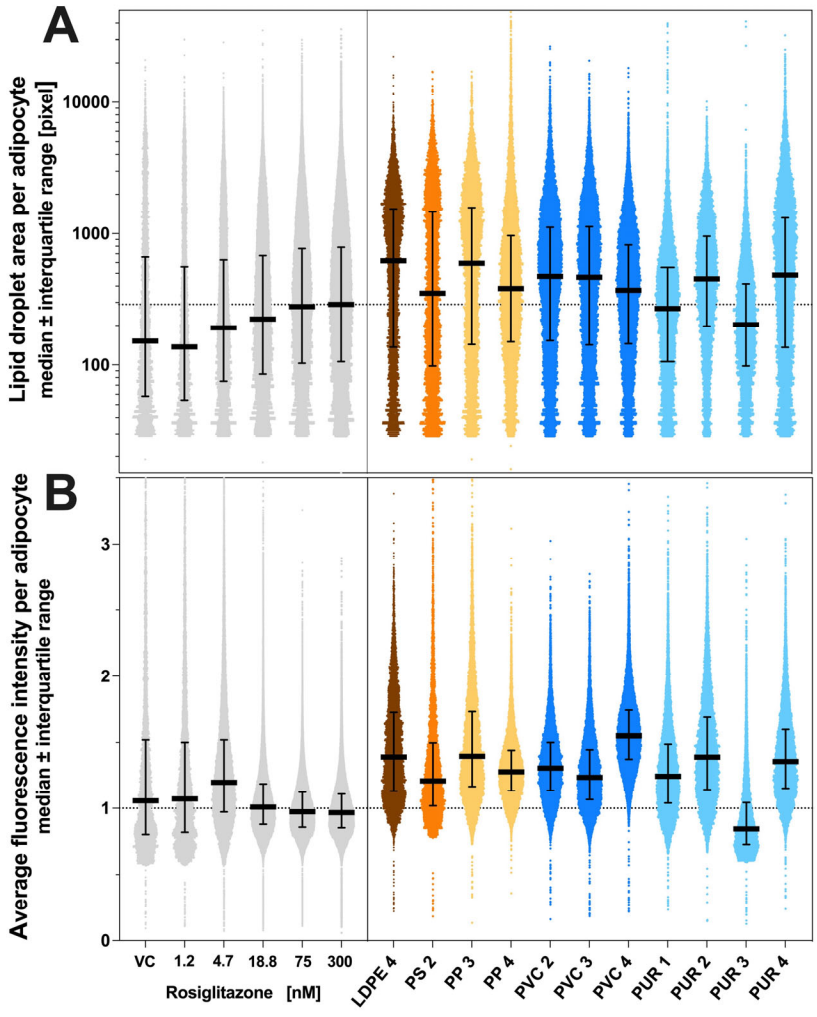
Data and materials availability

The raw mass spectral data can be accessed under DOI 10.5281/zenodo.4781257 (published after publication), bioassay data (SM Excel Tab. S3–10) and the CellProfiler pipelines can be found in the supplementary material.



686

687 **Fig. 1. Effect of rosiglitazone on (A) the adipocyte population and (B) the lipid droplet count**
688 **(pooled data from four experiments). Effect of plastic extracts on (C) the adipocyte**
689 **population and (D) the lipid droplet count in the highest noncytotoxic concentration.** The
690 highest noncytotoxic concentration (HNC) was 3 mg plastics well^{-1} except for PP 4 (1.5 mg plastic
691 well^{-1}) as well as PUR 2 and PUR 3 (0.75 mg plastic well^{-1}). VC = vehicle control, LOD = limit of the
692 detection, Rosi Max = maximal response of rosiglitazone.



693

694 **Fig. 2. (A) Size distribution of adipocyte population and (B) accumulation of triglyceride per**
695 **adipocyte in cells exposed to rosiglitazone (left) or the highest noncytotoxic concentration**
696 **of the eleven active plastic extracts (right).** Single-cell data from one experiment. Intensity data
697 is normalized on the mean of the highest rosiglitazone concentration (300 nM). VC = vehicle control.

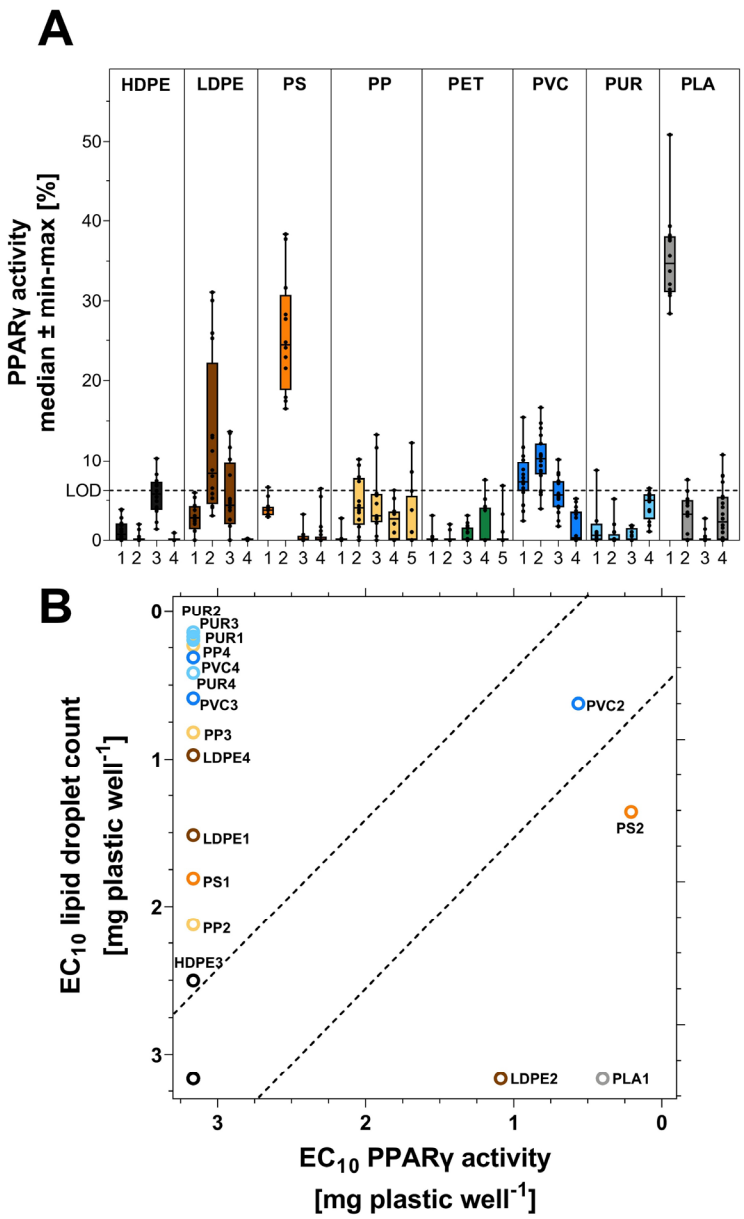


Fig. 3. (A) PPARγ activity induced by plastic extracts at the highest noncytotoxic concentration and (B) correlation of the EC_{10} of the PPARγ activity and lipid droplet count. The highest noncytotoxic concentration (HCN) was 1.5 mg plastic well^{-1} except for PP 4 (0.19 mg plastic well^{-1}), PS 2 and PP 3 (0.38 mg plastic well^{-1}) as well as PLA 1 and PVC 2 (0.75 mg plastic well^{-1}).

Table 1. Plastic products analyzed in this study, results of the nontarget chemical analysis and the tentatively identified metabolic disrupting chemicals (MDCs).

Sample	Plastic product	LC-QTOF-MS/MS (number of features)				Tentatively identified MDCs
		in sample	with MS2	ID score ≥40	% of MS2	
HDPE 1	refillable drinking bottle[a]	779	203	38	18.7	TPP
HDPE 2	yogurt drinking bottle[a]	107	34	7	20.6	
HDPE 3	bin liner	614	153	30	19.6	TPP
HDPE 4	shower gel bottle	164	50	16	32.0	EHDP
LDPE 1	lemon juice bottle[a]	241	66	20	30.3	EHDP
LDPE 2	plastic wrap[a]	1833	543	98	18.0	TPP
LDPE 3	freezer bag[a]	1603	416	62	14.9	TPP
LDPE 4	hair conditioner bottle	1702	544	89	16.4	allethrin, TPP
PS 1	yogurt cup[a]	447	96	12	12.5	TPP
PS 2	fruit tray[a]	1122	293	44	15.0	DPP, TPP
PS 3	vegetable tray[a]	308	63	11	17.5	
PS 4	plastic cup[a]	119	30	7	23.3	
PP 1	refillable drinking bottle[a]	1365	396	87	22.0	TPP
PP 2	yogurt cup[a]	1870	549	93	16.9	TPP
PP 3	gummy candy packaging[a]	3159	910	117	12.9	TPP
PP 4	handkerchief packaging	1798	519	85	16.4	TPP
PP 5	shampoo bottle	268	101	29	28.7	
PET 1	soft drink bottle[a]	148	55	18	32.7	
PET 2	yogurt cup[a]	179	51	12	23.5	
PET 3	oven bag[a]	647	159	30	18.9	
PET 4	vegetable tray[a]	695	182	20	11.0	
PET 5	shampoo bottle	375	89	11	12.4	
PVC 1	plastic wrap[a]	3655	1374	118	8.6	
PVC 2	place mat	2426	819	145	17.7	DPP, TPP
PVC 3	pond liner	1270	450	91	20.2	DINP, TPP
PVC 4	floor covering	2361	868	145	16.7	BBP, BPDP, DBP, DEHP, DINP, DPP, EHDP, TBEP, TOCP, TPP
PUR 1	scouring pad	5619	1773	216	12.2	EHDP, TPP
PUR 2	kids bath sponge	4521	1182	151	12.8	
PUR 3	acoustic foam	6242	2117	224	10.6	EHDP, TPP
PUR 4	shower slippers	1035	300	78	26.0	EHDP, TPP
PLA 1	yogurt cup[a]	2421	772	52	6.7	TPP
PLA 2	vegetable tray[a]	1983	672	40	6.0	
PLA 3	coffee cup lid[a]	N/A	N/A	N/A	N/A	
PLA 4	coffee cup lid[a]	2575	857	73	8.5	

[a] = food contact material, BBP = benzyl butyl phthalate, BPDP = tert-butylphenyl diphenyl phosphate, DBP = dibutyl phthalate, DEHP = bis(2-ethylhexyl) phthalate, DINP = di-iso-nonyl phthalate, DPP = diphenyl phosphate, EHDP = 2-ethylhexyl diphenyl phosphate, N/A = not analyzed, TBEP = tris(2 butoxyethyl) phosphate, TOCP = tri-o-cresyl phosphate, TPP = triphenyl phosphate