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Research Paper

Profile occurrences and in vitro effects of toxic organic pollutants in metal shredding facilities in Wallonia (Belgium)

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ABSTRACT

End-of-life vehicles and e-waste contain several hazardous substances that can contaminate the environment during treatment processes. Occurrences and adverse effects of toxic organic pollutants emitted from 3 shredder plants located in Wallonia, Belgium, were investigated by chemical and biological analyses of fluff, dust, and scrubbing sludge sampled in 2019. Site 1 showed the highest concentrations of chlorinated compounds in sludge with 7.5 ng/g polychlorinated dibenzo-dioxins/furans and 84.5 μg/g estimated total polychlorinated biphenyls, while site 3 led the brominated flame retardant levels in dust (53.4 μg/g). The level of polycyclic aromatic hydrocarbons was highest in the sludge samples, 78 and 71 μg/g for sites 2 and 3, respectively. The samples induced significant dioxin-like activities in murine and human cells at concentrations of around 0.01–0.1 and 0.5–1 ng (sample) per ml (medium), respectively, with the efficacy similar to 2,3,7,8-tetrachlorodibenzodioxin and EC_{50} values of around 1 and 10 ng/ml. The samples also displayed high estrogenic activities, already at 1 ng/ml, and several induced a response as efficient as 17β-estradiol, albeit a low androgenic activity. Shredder workers were estimated to be highly exposed to dioxin-like compounds through dust ingestion and dermal absorption, which is of concern.

1. Introduction

Impressive amounts of waste from end-of-life vehicles (ELVs) and electrical and electronic equipment (e-waste) are generated every year. For example, Europe produces 12 million tons of e-waste (2019) (Eurostat, 2021) and 8–9 million tons of ELVs every year (EC, 2018). Economically speaking, they are 'urban mines' which value billions USD (e.g. $57 billion USD in 2019 for e-waste worldwide) (Forti et al., 2020). In addition, the reuse of recycled materials helps to reduce net pollution during extracting and processing of raw materials. In fact, 42.5% of e-waste (2019) (Eurostat, 2021) and between 80% and 100% ELVs are collected and recovered or recycled in Europe (EC, 2018). Thus, recycling ELVs and e-waste is crucial to not only benefit the economy but also to protect the environment, which is in line with the circular economy package of the European Commission to reduce waste to a minimum, re-use, repair, refurbish, and recycle existing materials and products (EC, 2018).

Nevertheless, ELVs and e-waste contain several hazardous substances either as native components of the vehicle/equipment or released during the waste treatment, including toxic organic pollutants (typically persistent organic pollutants (POPs)) and heavy metals (Frazzoli et al., 2010). Consequently, the disposal sites and recycling areas can be highly contaminated and become the sources of their emission, especially if these activities are not carried out under appropriate regulation and control (Weber et al., 2008). POPs adversely affect human and environmental health. Once generated, they are persistent in the environment for a long period of time, and thus susceptible to biomagnification and long-range transportation (Walker, 2009; Yu et al., 2011). Although the production, usage, and release of POPs are supposed to be reduced and abolished according to the 2 international legally binding instruments, UNECE's "Protocol on Persistent Organic Pollutants of the Convention on Long-Range Transboundary Air

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Pollution" and "Stockholm Convention on Persistent Organic Pollutants", waste management is essential to control POP releases into the environment throughout their life-cycle (Potrykus et al., 2019).

In Europe, both ELVs and e-waste can be classified as metal waste, which is recycled by approved centers. After removal of hazardous fluids and hazardous or reusable parts, the dismantled metal scrap is subjected to material recycling mostly carried out in metal shredding facilities where they are fragmented into smaller pieces. The remaining (automotive) shredder residues ((A)SRs) presenting as a residual mixed fraction including the fluff collected by the air suction installation at the main shredder, are disposed of in landfills (Jody and Daniels, 2006; Sakai et al., 2014). (A)SRs often still contain several groups of POPs derived from mainly diffuse and undefined sources. Polychlorinated dibenzo-dioxins/furans (PCDD/Fs) and dioxin-like (dl-) polychlorinated biphenyls (PCBs) are generally the by-products of chlorine-containing production or combustion processes (Kulkarni et al., 2008) such as from synthetic leather (Sakai et al., 1998), diesel exhaust (Vermeulen et al., 2011), and other incomplete combustion products. Brominated flame retardants (BFRs) including polybrominated diphenyl ethers (PBDEs), are commonly incorporated in textile and plastic parts of vehicles and electrical and electronic equipment to reduce their inflammability (Abbasi et al., 2015; UNEP, 2010). All plastics containing BFRs must (theoretically) be removed based on the EU Directive 2012/19/EU on e-waste (EU, 2012) and 2000/53/EC on ELVs (EU, 2000). Besides, non dl-PCBs have been utilized intensively (1.5 million tons overall global production) in various commercial and chemical industries for a wide range of applications: dielectric fluids for capacitors and transformers, plasticizers in paints, flame retardants, diluents for pesticides, etc. Albeit banned in OECD countries, they can still be found e.g., in e-waste from past generations of electrical and electronic equipment with approximately 10% remaining in the environment today (Reddy et al., 2019).

Unintentional generation of POPs can also occur during thermal treatment processes. For example, copper wires combined with halogen compounds lead to the formation of PCDD/Fs (Buekens and Zhou, 2014). De novo synthesis and decomposition of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs), mixed halogenated dioxins/furans (PXDD/Fs), and PCBs are recorded during thermal processes (Ishikawa et al., 2007; Van Caneghem et al., 2014; Weber and Kuch, 2003). Incomplete combustion of carbonaceous materials at high temperature can also generate polycyclic aromatic hydrocarbons (PAHs) that are classified as some of the most spread mutagenic and carcinogenic environmental pollutants (Yu, 2002). Other groups of toxic organic pollutants that also potentially contaminate shredder waste such as phthalates, per- and polyfluoroalkyl substances (PFASs), polychlorinated naphthalenes (PCNs), plasticizers, and short chain chlorinated paraffins (SCCPs), have not received much attention.

While several studies have been done on (A)SRs, few studies have assessed the deposition of polluted dusts emitted from shredding plants. For example, PBDEs were found in the vicinity of the shredding plants at 50-time higher levels compared to residential areas in Australia (Hearn et al., 2012), while in USA, people were found to be exposed to 40,000 pg PDBEs/kg body weight/day through inhalation of indoor air in a location adjacent to an electronic shredding equipment (Cahill et al., 2007). Sludge accumulating in wet shredders when cleaning the contaminated wastewater coming from the pre-wetting of the shredder materials, has to be disposed of (2015) and subjected to other waste treatment processes. The data about POP contamination in sludge generated by shredding activities are scarce.

In POP monitoring, effect-based approaches such as in vitro cell-based assays are particularly of interest, since they offer low-cost, high-throughput, and alternative analytical methods for trace detection of POPs (Reddy et al., 2019). Bioassays are also indicatives of physiology-based responses. Receptor activation or inhibition can indicate the initiation of defense mechanisms, and thus the presence of contaminants in the sample even at low doses. Moreover, they allow the study of the joint biological effect of all active chemicals in a sample as a whole (Escher et al., 2018), regarding that the chemicals can interact with one another and the effects of many chemicals at concentrations lower than their lowest observed effect concentrations can be significant in the mixture (Doan et al., 2019; Thrupp et al., 2018). Indeed, a combination of instrument- and bioassay-based assessment is necessary for an adequate evaluation of the total effect of all contaminants in the complex matrices of shredder waste (Nguyen et al., 2013). In addition to carcinogenesis, exposure to POPs can cause endocrine disruption which can be severe in both short and long terms even at very low doses during vulnerable periods (development and reproduction) (Demeneix and Slama, 2019). These toxic effects of POPs can be evaluated by their interfering or interacting with several transcription factors including the master xenobiotic aryl hydrocarbon receptor (AhR) and the steroid (i.e. androgen (AR) and estrogen (ER)) receptors (Balaguer et al., 2019; Demeneix and Slama, 2019).

About 3.2 million tons of metal scrap are recycled annually in Belgium. François et al. (2004) found 16–29 pg PCDD/Fs and 43–137 pg PCB-126 TEQ/m^2.day when assessing their deposition near 4 Flemish (Dutch speaking part of Belgium) metal shredding facilities (2003–2004). Dufour et al. (2020) measured the concentrations of 17 PCDD/Fs, 18 PCBs, and 24 PBDEs in fallout dust collected in the vicinity of 3 shredding plants located in Wallonia (French speaking part of Belgium) from 2017 to 2019. The results showed high levels of total PCDD/Fs (6.2 pg TEQ/m^2.day), PCBs (246.5 ng/m^2.day) and PBDEs (253.8 ng/m^2.day for BDE-209) compared to those observed in remote, rural or even some urban areas around the world, and were similar to those measured in other heavily industrialized areas.

The aims of the present study were to further investigate the occurrences and adverse effects of toxic organic pollutants emitted from the 3 shredder plants in Wallonia as in Dufour et al. (2020): (i) measure the occurrences of several toxic organic pollutants (PCDD/Fs, PXDD/Fs, PCBs, BFRs, PAHs, phthalates, PFASs, PCNs, SCCPs, plasticizers, and others) in fluff, dust, and sludge samples, (ii) investigate in vitro toxic effects of the samples on 7 luciferase reporter gene cell lines for dioxin, estrogenic, androgenic, and oxidative stress responsiveness, and (iii) identify the contributions of compounds/groups for the chemical compositions and bioactivities. The study provided the profiles of the occurrences and toxic potentials of these hazardous compounds in shredder waste, enabling further understanding of their toxicity and risk assessments.

2. Materials and methods

2.1. Sampling and sample processing

Three different kinds of samples were collected from the 3 shredding plants in June (site 1) and December (sites 2 and 3) 2019: fluff (1F, 2F, and 3F) as the light fraction separated through air classification from the shredded bulk, dust on the ground (1D, 2D, and 3D), and sludge from gas scrubbing (1S, 2S, and 3S). Soil sampled in the Walloon countryside, Havelange (50.385751, 5.153126), in Belgium, July 2020, was used as the control (CT). These samples were uniformly collected by a mean of a clean scoop into a glass jar from various representative places. They were then dried, ground and homogenized by SGS Belgium NV, Antwerp, Belgium ready for chemical and biological analyses. While sites 2 and 3 belong to the same company, which recycle both ELVs and e-waste, site 1 is from a different company working only with ELVs.

2.2. Chemical analyses

The samples were analyzed for the content of several groups of organic pollutants (PCDD/Fs, PXDD/Fs, dl- and non dl-PCBs, PBDEs, PAHs, phthalates, PFASs, PCNs, plasticizers, and SCCPs) also by SGS Belgium NV under DIN EN ISO/IEC 17025:2005 accreditation. PAHs were not analyzed for site 1 (except benzo(a)pyrene (BaP)) and CT.

2.3. Biological analyses

2.3.1. Extraction

We used a sequential extraction method with increasing polarity of the solvents (hexane and acetone (Sigma Aldrich, Missouri, USA)) to extract high-to-lower hydrophobic compounds. A portion of each sample (around 2 g) was extracted using sonication with hexane (20 ml; 20 min), followed by 10 min shaking. The supernatant was collected after centrifugation at 2500 rpm for 10 min at 4 °C. Then, 20 ml fresh hexane was added to the precipitated faction for an additional extraction of the solid residue, which was repeated twice. The 3 hexane extracts were then pooled together to make the hexane fraction. The same extraction procedure was applied to the dry residue obtained after hexane extraction using acetone to make the acetone fraction. After that, the hexane and acetone extracts were concentrated using a Turbovap to 1 ml and further blown down under a gentle nitrogen gas stream. At the point of dryness, 100 μL dimethyl sulfoxide (DMSO, Acros Organics, Molinons, France) was added to obtain an equivalent concentration of 0.02 g sample/μL DMSO. Each sample was extracted in duplicate. Procedural blanks for hexane and acetone factions were also included. We used the whole mixture approach without acid-cleanup since the cleanup may affect the mixture composition e.g. due to a non-quantitative removal of PAHs (Lamoree et al., 2004).

2.3.2. In vitro cell-based assays

Seven genetically modified cell lines expressing the luciferase reporter gene were used for analyzing bioactivities of samples from sites 2 and 3 due to their full chemical profiles. The assays measure the transcriptional activity of the receptors through the expression of a luciferase reporter gene under the control of a recombinant promotor containing the receptors' responsive elements. The induction (agonism) or inhibition (antagonism) of the transcriptional activity of the receptors responding to stimuli (chemicals) is the basis of the assays.

Four dioxin responsive cell lines: 2 commercialized murine cell lines i.e. rat hepatoma DR-CALUX (BioDetection System, Amsterdam, The Netherlands) (Aarts et al., 1995) and mouse hepatoma DR-EcoScreen (National Institutes of Biomedical Innovation, Health, and Nutrition JCRB cell bank) (Kojima et al., 2018), and 2 human cell lines i.e. hepatoma DR-HepG2-Luc and mammary gland DR-T47D-Luc (both produced on site in ULiege, Belgium) (Van der Heiden et al., 2009), were used to compare the AhR transcriptional activity between species (murine and human) and tissues (hepatoma and mammary gland). Human breast carcinoma VM7Luc4E2 (donated by Professor Michael Denison, University of California, USA) (OECD, 1995) and Chinese hamster ovary AR-EcoScreen GR KO (also from JCRB cell bank) (Zwart et al., 2017) addressing respectively the estrogen and androgen responsiveness, were also used.

Protocols for cell culture and the procedure of the reporter gene assays for each cell line were described in detail elsewhere in Table 1. Briefly, after the cells were seeded in white-walled clear-bottomed 384 well microplates (Corning, Acton, MA, USA) for 24 h and reached 90% cell confluence, they were then exposed to a 9-point half-fold dilution series of hexane or acetone extracts. For quality control, standard curves (Fig. S1) of the reference chemicals (all purchased from Sigma) along with the blank vehicle DMSO were performed on each plate. In addition to the agonistic tests, co-exposure or antagonistic tests in which the cells were co-exposed to both the same dilution series of the extracts and the reference chemical at its EC_{80} (Table 1), were also performed. All cells were exposed to the extracts for 24 h in DMEM without phenol red (Lonza, Walkersville, MD, USA) supplemented with 10% charcoal stripped fetal bovine serum (Greiner, Kremsmünster, Austria) to eliminate natural steroid activators. After exposure, the cells were lysed by lysis buffer containing Triton X100 (Sigma Aldrich). Then, luciferin (Promega, Madison, WI, USA) and ATP (Roche Diagnostics, Rotkreuz, Switzerland) were added to the cell lysate for luminescence reactions.

The luminescence signal was measured by a Tecan Spark multimode reader (Tecan Trading AG, Switzerland) as relative light units. The final concentration of DMSO was 0.2% and 0.3% for the agonistic and co-exposure tests, respectively. The maximum tested concentrations were 10 (hexane extracts) and 20 (acetone extracts) ng/ml (sample/medium). All tests were performed in at least 2 independent experiments, each in quadruplicate. The cytotoxicity was examined by visually inspecting the cell morphology and density under the microscope before cell lysis. In addition, the Nrf2 reporter gene assays (MCF7-AREc32 obtained from Ximbio, London, UK) (Xiu et al., 2006) were also carried out to test for any oxidative stress due to cell damage when the cells were exposed to the extracts (He et al., 2020).

2.4. Data analysis

2.4.1. Chemical analyses

The measurement uncertainty of chemical analyses was 20% for PCDD/Fs, PXDD/Fs, and PCBs, 30% for BFRs, and around 40% for the other compounds. Results for some compounds having their range out of the linearity of the plot (marked as * in Table S1) were still quantitative since the detector was not saturated.

Toxic equivalency (TEQ) relates the toxicity of a mixture/sample to that of a well-characterized chemical based on the Toxicity Equivalency Factor (TEF_i) and the concentration (C_i) of each component (i) in the mixture (Eq. (1)) (therefrom TEQ-chem). TEQ was calculated for PCDD/Fs and dl-PCBs according to the TEFs of (Van den Berg et al., 2006), and for PAHs from TEFs established by Machala et al. (2001) using induction equivalency factor from EC_{50} 2,3,7,8-tetrachlorodibenzodioxin (TCDD), 24 h exposure in DR-CALUX cells. Naphthalene, acenaphthylene, acenaphthene, and fenanthrene were not considered due to missing TEFs. For TEQ calculations and data analyses, concentrations below the limit of quantitation (LOQ) were assigned as the values of one-half of the LOQ (medium-bound approach) (Bergstrand and Karlsson, 2009) (marked as ~ in Table S1).

$$\mathrm{TEQ-chem} = \sum(\mathrm{TEF_i} \times \mathrm{C_i}) \quad (1)$$

2.4.2. In vitro cell-based assays

Each replicate in the duplicates of the extracts was analyzed indi-

Table 1
Characteristics of the 7 reporter gene cell lines and the co-exposure EC_{80} concentrations.

No	Names	Original cell line	Receptor	Reference	EC_{50} (pM)	FI_{max} (fold)	EC_{80} (pM)
1	DR-CALUX	H4IIE: rat hepatoma	AhR	TCDD	10	13	19
2	DR-EcoScreen	Hepa1c1c7: mouse hepatoma	AhR	TCDD	6.2	5	10
3	DR-HepG2-Luc	HepG2: human hepatoma	AhR	TCDD	667	12	1400
4	DR-T47D-Luc	T47D: human breast carcinoma	AhR	TCDD	124	9	350
5	AR-EcoScreen	CHO-K1: Chinese hamster ovary	AR	DHT	89	6	500
6	VM7Luc4E2	MCF7: human breast carcinoma	ER	E2	2	10	25
7	MCF-7/AREc32	MCF7: human breast carcinoma	Nrf2	tBHQ	–	10 (at 15 μM)	–

EC_{50}: Concentration causing half-maximal response of the reference chemical. FI_{max}: maximum fold induction of the response of the reference chemical compared to the response of the vehicle DMSO. EC_{80}: Co-exposing concentration of the reference chemical used in the antagonistic/co-exposure tests. TCDD: 2,3,7,8-tetrachlorodibenzodioxin, DHT: dihydrotestosterone, E2: estradiol, tBHQ: tert-butylhydroquinone.

vidually in the bioassays, the result for each was then pooled together as a single data point (n = 4), considering both variations in chemical and biological analyses. After DMSO subtraction (R_{DMSO}), relative responses (RR_{AG}/RR_{AN}) (%) were presented as the final results, i.e. percentages of the cell response to the extracts compared to the maximum response of the cells to the reference chemical ($R_{ref\text{-}max}$) on the same plate for agonistic activities (Eq. (2)), or to spike-in EC_{80} of the reference chemical ($R_{ref\text{-}EC80}$) for antagonistic activities (Eq. (3)). In the antagonistic/co-exposing tests, the cell response to the spiked-in EC_{80} (100%) was subtracted. Antagonistic activities were shown as negative values, while any agonistic activity of the extracts on top of the spiked-in reference chemical was presented as positive values.

$$RR_{AG} = \frac{R_{AG} - R_{DMSO}}{R_{ref-max} - R_{DMSO}} \times 100 \quad (2)$$

$$RR_{AN} = \frac{R_{AN} - R_{DMSO}}{R_{ref-EC80} - R_{DMSO}} \times 100 - 100 \quad (3)$$

A non-linear regression of 4-parameters: bottom (B), top (T), hill-slope (H), and half-maximum effect concentration (EC_{50} or IC_{50}) was used to fit the dose-response curves (Graphpad PRISM software, version 9). The relative responses RR_{AG} (Eq. (4)) or RR_{AN} (Eq. (5)) induced by concentration x of the extracts for agonistic or antagonistic tests were formulated.

$$RR_{AG(x)} = B + \frac{x^H(T - B)}{x^H + EC_{50}{}^H} \quad (4)$$

$$RR_{AN(x)} = B + \frac{T - B}{1 + \frac{x^H}{IC_{50}{}^H}} \quad (5)$$

Maximum relative responses (RR_{AGmax} and RR_{ANmax}) were the maximum responses of the cells to the highest tested concentration of the samples respectively for the agonistic and antagonistic/co-exposing tests.

Bioanalytical equivalent (therefrom TEQ-bio) calculated from biological activities, relates the toxicity of a mixture/sample to a well-characterized chemical by comparing its response to that of the reference chemical. The concentration of the extract used for TEQ-bio calculation was chosen as the maximum or the highest concentration which gave a response lying ideally between 20% and 70% of the maximal response of the reference chemical (Fig. S2) (Elskens et al., 2011). The TEQ concentration (x_{TEQ}) of the extract causing the same response (RR_{AG}) as the reference chemical was calculated by inverting the regression model described by Eq. (4).

2.4.3. Chemical and biological references

Ordination by canonical correspondence analysis (CCA) is usually used to explain how biological responses are linked to environmental variables mostly in ecological data-sets (Ter Braak and Verdonschot, 1995). In this study, CCA was applied to elucidate the roles of the compounds for the chemical compositions and bioactivities of sites 2 and 3. To select chemical explanatories, heatmap cluster and principal component analyses (PCA) were used. While heatmap depicts the observed data without any pre-processing, PCA helps reduce the dimensionality but retains most of the variations in data-set (Jolliffe, 2002). In this study, chemicals were grouped according to their concentrations by heatmap cluster using the full set of chemicals (Table S1) by R statistical software with scale normalization. PCA was used to confirm the representativeness for the separation of the selected groups. Then, they were used to correlate with the bioactivities to identify the roles of each group on the activities of the samples. CCA and PCA analyses were performed in Past 4.03 (Hammer et al., 2001) with log transformation for the concentrations of the chemicals.

3. Results

3.1. Profile occurrences of different hazardous compound groups

Data for PCNs, PFASs, phthalates, hexabromobiphenyl, and hexachlorobutadiene were mostly below LOQs, thus they were subsequently excluded from further analyses. Table 2 presents the absolute concentrations of several groups: PCDD/Fs, PXDD/Fs, dl-PCBs, estimated total PCBs, PBDEs, total BRFs, and total PAHs in fluff (F), dust (D), and sludge (S) samples collected from the 3 shredding plants (sites 1, 2, and 3) and in the soil from the control site (CT) in Belgium. The name and concentration of each compound is given in Table S1. In general, the contamination level of samples from these shredding plants was several orders of magnitude higher than that (mostly below the LOQs) of the control soil. While site 1, especially the sludge 1S, showed the highest levels of chlorinated contamination (3–4 folds) (Fig. 1A–C), BFRs was most abundant in the dust of site 3 (3D) (> 3 folds) (Fig. 1D). For PAHs, a common contamination pattern was observed: fluff < dust < sludge (Fig. 1E).

This study quantified 17 PCDD/Fs, 18 PCBs, and 8 groups of PXDD/Fs for chlorinated contamination. Sample 1S had the highest PCDD/F concentration, 7.5 ng/g, while the other sludge samples showed much lower figures of 0.38 (2S) and 0.65 (3S) ng/g. In fluff and dust samples, the level was around 2 ng/g (Table 2, Fig. 1A). The levels of PCDDs were higher than those of PCDFs by 4–8 folds, in which OCDD accounted for more than 70% of the total weight of PCDD/Fs (Fig. 1A, Table S1). However, PCDDs and PCDFs had similar weight in dust from sites 1 and 2 with 2,3,7,8-TCDF and OCDF (both 22%), respectively being more pronounced. Samples 2F, 3F and 3D showed higher levels of PXDD/F contamination (14, 6.8, and 11 ng/g, respectively) than the others (Table 2) due to 90% contribution of hepta-furans (Table S1).

The PCB contamination pattern was quite similar among these samples (Table S1). For the 12 dl-PCBs, the concentrations of PCB-118 and PCB-105 were highest, contributing for about 55% and 25% (Fig. 1B). For 6 non dl-PCBs, PCB-28 + PCB-31 (analyzed together as a sum of both congeners) contributed most (30–50%), although 1S also displayed relatively high portions of the other non dl-PCBs (Fig. 1C). According to the European Guideline DIN EN 12766-2 (2001), the total PCB content was estimated by multiplying by 5 the sum of the 6 non dl-PCBs (5x$\sum$6PCBs DIN) (Table 2). Similar with PCDD/Fs, the highest level of the estimated total PCBs (84.5 μg/g) was found in the sludge 1S, while the other samples gave a lower figure (around 10 μg/g) (Table 2).

Eight PBDEs and 3 other BFRs were selected for quantification in this study. The highest BFR level was detected in dust sample 3D with 53.4 μg/g, which was 3 folds the second highest (sludge sample 1S, 17.7 μg/g) (Fig. 1D, Table 2). BDE-209 contributed already for around 80% of the qualified BFRs, except for the fluff 3F, where it represented only 35% of the total, and 28% came from hexabromocyclododecane (HBCD) (Fig. 1D, Table S1).

Only BaP was measured in site 1 while sites 2 and 3 were analyzed for 16 PAHs listed in the priority pollutant list of the US EPA (1982). Contrary to sample-borne chlorinated and brominated contaminants whose profiles seemed specific of the sampling sites, PAH emissions appeared more dependent on sample types. The highest levels of PAHs were observed for the sludge with 78 and 71 μg/g for sites 2 and 3, respectively, followed by the dust with 26.7 and 17.8 μg/g, and the least was for the fluff with 8.4 and 16.8 μg/g. The dominant group constituting 10–30% of the total PAHs included phenanthrene, fluoranthene, pyrene, and benzo(g,*h*,i)perylene at a lesser extent (Fig. 1E, Table S1).

3.2. Bioactivities

Neither cytotoxicity by morphology nor stress response (no activities in the MCF7-AREc32, data not shown) was observed for any extract at any tested concentration. The full dose response curves of the extracts are presented in Figs. S3–S5, while Table 3 gives information about the

Table 2
Profile occurrences and toxic equivalency (TEQ) of different hazardous compound groups in shredder waste.

Samples	1F	1D	1S	1DF[a]	2F	2D	2S	2DF[a]	3F	3D	3S	3DF[a]	CT
PCDD/Fs (pg/g)	2423	2268	7498	ND	1575	1478	376	ND	2809	2861	651	ND	~109
PXDD/Fs (pg/g)	1198	2479	5634	ND	14,268	2670	1054	ND	6750	10,577	398.7	ND	~76
dl-PCBs (ng/g)	321	740	3959	42	427	136	329	53	341	278	312	29.1	~0.7
Estimated total PCBs (ng/g)	10,303	20500	84,495	551	14,187	4438	11,599	1246	13,957	11,068	9222	502	~29
PBDEs (ng/g)	9548	4100	16,232	662.4	ND	735	ND	1012.8	4109	51,238	1116.8	431.2	~24.3
BFRs (ng/g)	10,718	4470.1	17,692	ND	ND	1004.7	ND	ND	6539	53,448	1394.3	ND	~46.8
PAHs (ng/g)	16,000*	1500*	8300*	ND	8428	26,660	78,000	ND	16,765	17,795	70,960	ND	ND
PCDD/F/dl-PCBs (WHO pg TEQ/g)[b]	66.2	314.9	627.2	10.7	85	68.7	51.1	23	75.9	65.2	47.7	10.7	~8.3
PAHs (DR-CALUX pg TEQ/g)[c]	1442*	135*	748*	ND	744	2570	6863	ND	1466	1424	5479	ND	ND
TEQ-chem (pg TEQ/g)	1508	450	1375	ND	829	2638	6914	ND	1542	1489	5527	ND	ND
TEQ-bio (DR-CALUX pg TEQ/g)	ND	ND	ND	ND	9498 ± 2646	10,851 ± 6598	7050 ± 904	ND	3507 ± 727	5627 ± 1600	323 ± 119	ND	1760 ± 780
TEQ-bio (DR-EcoScreen pg TEQ/g)	ND	ND	ND	ND	11,885 ± 3595	12,762 ± 3609	10,112 ± 2986	ND	6852 ± 1857	9258 ± 3236	658 ± 285	ND	1510 ± 510
TEQ-bio (DR-HepG2-Luc pg TEQ/g)	ND	ND	ND	ND	52,851 ± 13,425	49,196 ± 14,791	37,870 ± 8894	ND	15,925 ± 7126	43,679 ± 15,772	21,351 ± 6460	ND	8564 ± 5310
TEQ-bio (DR-T47D-Luc pg TEQ/g)	ND	ND	ND	ND	23,953 ± 7041	21,722 ± 4367	13,045 ± 2458	ND	6661 ± 2690	11,011 ± 2738	15,849 ± 4466	ND	1834 ± 1624
TEQ-bio (VM7LucE2 pg E2-eq/g)	ND	ND	ND	ND	161 ± 70	69 ± 27	148 ± 63	ND	122 ± 38	80 ± 17	34 ± 16	ND	7 ± 2

(–) The values which were lower than detection limits were assigned as half of the limit of quantitation (LOQ/2)- Medium bound.
ND: Not determined.
Estimated total PCBs = 5x∑6PCBs DIN (ng/g), PCB-28 + PCB-31, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180.
*Only benzo(a)pyrene was measured in the sludge sample of site 1 (1S).
F: fluff, D: dust, S: sludge, DF: depositional fluxes*(/m^2.day), and CT: control soil.
[a] Data were cited from Dufour et al. (2020).
[b] Toxicity Equivalency Factor (TEF) from (Van den Berg et al., 2006).
[c] Toxicity Equivalency Factor (TEF) from Machala et al. (2001).

RR_{AGmax}/RR_{ANmax}, EC_{50}, and R^2 of these curves.

All hexane extracts induced similar agonistic activities in the 2 murine cells significantly ($RR_{AG} > 10\%$) at already 0.01–0.1 ng sample/ml medium and EC_{50} < about 1 ng/ml with $RR_{AGmax} = 80–120\%$, i.e. similar to the maximum cell responses to TCDD, except a lesser extent for the sludge 3S in DR-EcoScreen ($RR_{AGmax} = 66 \pm 13\%$) and DR-CALUX ($RR_{AGmax} = 34 \pm 12\%$) (Table 3, Figs. S3A and S3E). Acetone extracts also induced strong agonistic activities, and DR-EcoScreen gave higher responses (RR_{AGmax} = 60–100%) compared to DR-CALUX's (RR_{AGmax} = 25–60%) (Table 3, Figs. S3C and S3G). In co-exposure tests, hexane extracts still kept their agonistic behaviors with higher responses in DR-CALUX than in DR-EcoSreen (Table 3, Figs. S3B and S3F). They induced an additive response on top of the co-exposing TCDD EC_{80} shown by positive RR_{ANmax} values. Although most of the RR_{ANmax} were lower than the corresponding agonistic RR_{AGmax}, samples 2 F, 2D, 3D, and 3 S displayed higher values for RR_{ANmax} ($116 \pm 16\%$, $134 \pm 28\%$, $134 \pm 8\%$, and $52 \pm 18\%$, respectively) than for RR_{AGmax} ($112 \pm 22\%$, $117 \pm 15\%$, $124 \pm 15\%$, $34 \pm 12\%$) in DR-CALUX. By comparison, while the acetone extracts kept their agonisms when co-exposing with EC_{80} TCDD in DR-EcoScreen, these effects were less intense in DR-CALUX (Table 3, Figs. S3D and 3SH).

Hexane extracts also showed agonistic activities in the 2 human cell lines, significantly ($RR_{AG} > 10\%$) at already 0.5–1 ng/ml and EC_{50} < about 10 ng/ml (Table 3, Figs. S4A and S4E). Higher activities (high efficacy) and steeper slopes (high potency) were observed for the mammary gland carcinoma DR-T47D-Luc ($RR_{AGmax} = 90–133\%$, except $RR_{AGmax} = 31 \pm 6\%$ for CT) compared to the hepatoma DR-HepG2-Luc ($RR_{AGmax} = 20–50\%$). Interestingly, the extracts antagonized the activity of EC_{80} TCDD in DR-HepG2-Luc ($RR_{ANmax} = -22\%$ to -55%), while elevating it in DR-T47D-Luc ($RR_{ANmax} = 23–75\%$). No effect was seen in antagonistic tests for CT in both cell lines and for 3F in DR-T47D-Luc (Table 3, Figs. S4B and S4F). Acetone extracts displayed a similar pattern with the hexane in the 2 cell lines but to a much lesser extend (Table 3, Figs. S4C, D, G, and H).

A relatively low androgenic activity ($RR_{AGmax} = 8–37\%$) was seen for all extracts. While most of them still remained in the co-exposing tests, anti-androgenic activities were recorded for samples 2F and 2D with $RR_{ANmax} = -38 \pm 9\%$ and $-40 \pm 6\%$, respectively for the hexane extracts (Table 3, Figs. S5E–H). Meanwhile, both hexane and acetone extracts had high estrogenic activities, significantly at already 1 ng/ml (Figs. S5A and S5C). Samples 2F and 2D exhibited the strongest RR_{AGmax} = $67 \pm 11\%$ and $54 \pm 11\%$, EC_{50} = 4.6 and 2.8 ng/ml for hexane extracts, and $RR_{AGmax} = 88 \pm 5\%$ and $89 \pm 5\%$, EC_{50} = 5 and 3.2 ng/ml for acetone extracts, respectively (Table 3). Hexane extract from sample 3D induced a strong estrogenic activity ($RR_{AGmax} = 62 \pm 10\%$, EC_{50} = 12 ng/ml), while acetone extract from sample 3F displayed a high $RR_{AGmax} = 94 \pm 5\%$, EC_{50} = 3 ng/ml. Nevertheless, a moderate anti-estrogenic activity was recorded only for hexane extracts (RR_{ANmax} = −17% to −36%), except 3S. Indeed, samples 3S and CT were generally the least active or induced no significant effects in the 2 steroid reporter cells (Table 3, Figs. S5). Note that CT hexane extract showed $RR_{AGmax} = 12 \pm 3\%$ in AR-EcoScreen, but it trigged a stronger activation ($RR_{ANmax} = 67 \pm 22\%$) when cells were co-exposed to DHT EC_{80}.

3.3. Toxic equivalents (TEQs)

Due to its high content of PCDD/Fs and dl-PCBs, sample 1S (627.2 pg PCDD/F/dl-PCB WHO TEQ/g dry weight) followed by sample 1D (314.9 TEQ pg/g), displayed nearly 10-fold WHO TEQ higher than the other samples (around 65 pg TEQ/g) (Table 2). PCDD/Fs accounted for 20–30% of WHO TEQs, except in sample 2D where they presented 64%. Hence, 70–80% of WHO TEQs were from dl-PCBs with 55–60% from PCB-126 and nearly 10% from PCB-118 (Fig. 2A1).

However, PAHs were the major contributors for the TEQ-chem. The PAH TEQs were up to 2 orders of magnitude higher than WHO TEQs for samples from sites 2 and 3 (Fig. 2A2, Table 2). More than 50% of the

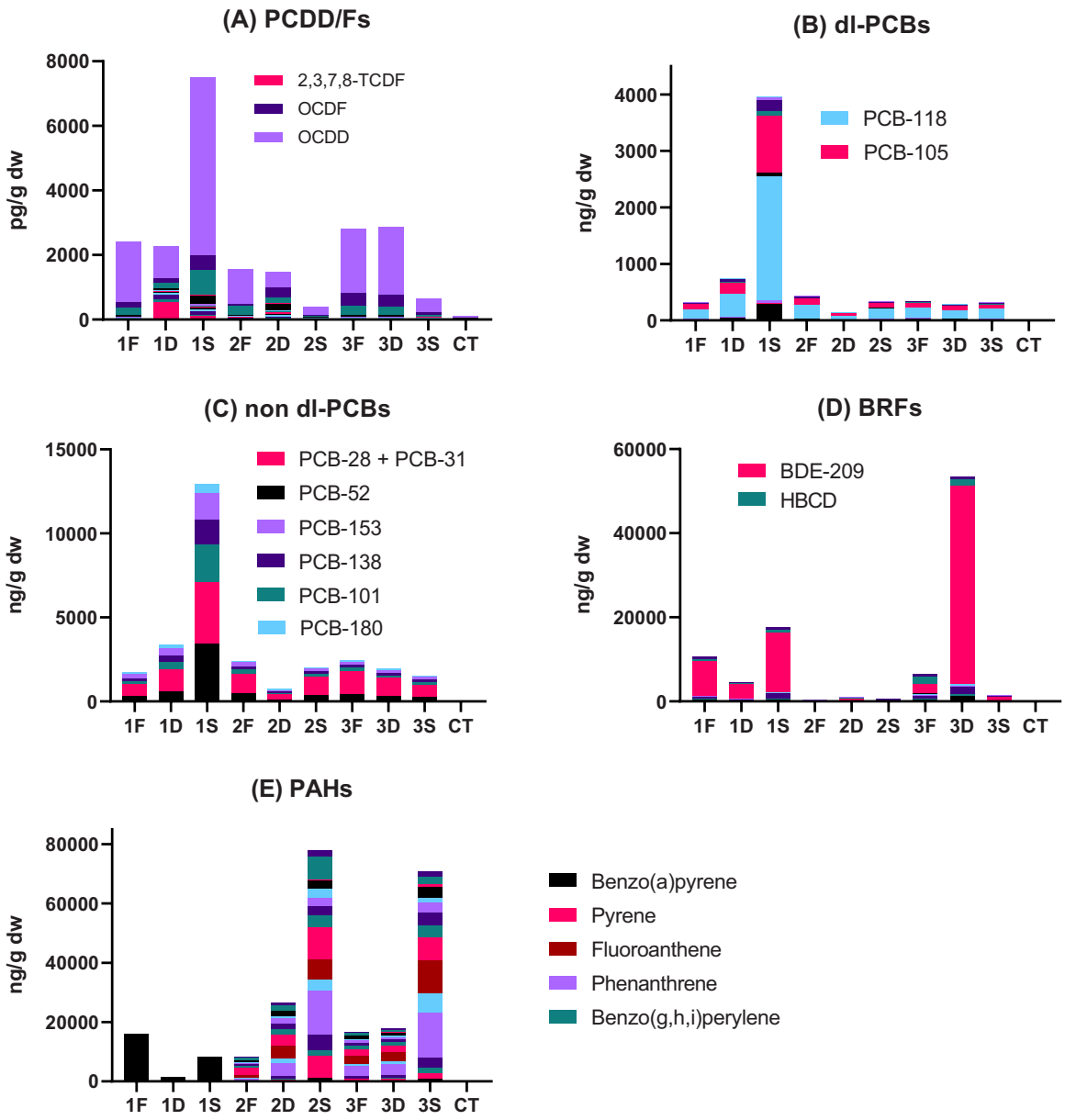

Fig. 1. Profile occurrences of different hazardous groups measured in fluff (F), dust (D), and sludge (S) samples collected from the 3 different shredding plants (sites 1, 2, and 3) and in soil from the control site (CT). (A) PCDD/Fs (polychlorinated dibenzo-dioxins/furans), (B) dl-PCBs (dioxin-like polychlorinated biphenyls), (C) non dl-PCBs (non dioxin-like polychlorinated biphenyls), (D) BRFs (brominated flame retardants), (E) PAHs (polycyclic aromatic hydrocarbons).

PAH TEQs were from benzo(k)fluoroanthene, whereas around 10–20% were from dibenzo(ah)anthracene and indeno(1,2,3-cd)pyrene. Interestingly, with only BaP detected, PAH TEQ of site 1 was high with the fluff 1F (1442 pg TEQ/g) displaying either similar PAH TEQ with the fluff and dust samples or 5-time lower than the sludge from sites 2 and 3 (Fig. 2A2, Table 2).

Fig. 2(B–F) shows that the dl-activities were due to hexane extracts, while acetone extracts also played a significant role in estrogenic-like activities with about 50% contribution for samples 2F, 2D, and 3F. TEQs from bioassays (TEQ-bio) of dl-compounds that induced AhR agonisms, exhibited several folds higher with different responding patterns than the TEQ-chem (Fig. 2). The 2 human cells gave 2- to 3-fold higher TEQ-bio values than the 2 murine cells, in the order DR-EcoScreen = DR-CALUX < DR-T47D-Luc < DR-HepG2-Luc. For example, while the 2 murine cells gave similar TEQ-bio of 9.5 ± 2.6 and 11.9 ± 3.6 ng TEQ/g sample for 2F, respectively for DR-CALUX and DR-EcoScreen (Fig. 2B-C), higher TEQs were seen for the 2 human cells, 24 ± 7 and 52.9 ± 13.4 ng TEQ/g, respectively for DR-T47D-Luc and DR-HepG2-Luc (Fig. 2D-E). TEQ-bio figures for samples of site 2 were nearly 2 folds higher than those of samples from site 3. The control sample CT displayed the lowest dl-activities; however, the sludge from site 3 (3S) which had high levels of PAHs, showed even lower TEQ-bio than CT in the 2 murine cells despite a rise to higher levels in the 2 human cells (Fig. 2B–E).

3.4. Relationship between chemical concentrations and biological activities

Based on heatmap and hierarchical clustering analysis (Fig. S6), we were able to separate 8 groups of chemicals distinctively for the shredder plants. The PCA diagram (Fig. 3A) illustrates the 8 selected groups (chemical variables) explaining the relationship among the samples of sites 2 and 3 and the contributions of each group for the ordination. The PCA ordination was in agreement with the heatmap of

Table 3
Bioactivities of hexane and acetone extracts from shredder waste samples of sites 2, 3 and the control soil.

Cell line	Parameters	2 F	2D	2 S	3 F	3D	3 S	CT	2 F	2D	2 S	3 F	3D	3 S	CT
		Hexane extract - Agonistic tests							Acetone extract - Agonistic tests						
DR-EcoScreen	RR_{AGmax}	81 ± 19	113 ± 17	82 ± 8	89 ± 20	110 ± 22	66 ± 13	82 ± 10	83 ± 17	106 ± 28	60 ± 8	74 ± 4	78 ± 7	72 ± 18	54 ± 5
	EC_{50}	0.046	0.11	0.14	0.25	0.39	–	1.06	–	4.13	5.06	13.53	3.21	2.38	3.18
	R^2	0.92	0.93	0.92	0.94	0.91	0.89	0.87	0.91	0.94	0.93	0.97	0.96	0.9	0.68
DR-CALUX	RR_{AGmax}	112 ± 22	117 ± 15	114 ± 6	88 ± 22	124 ± 15	34 ± 12	71 ± 4	35 ± 6	42 ± 22	26 ± 4	45 ± 6	57 ± 8	45 ± 9	25 ± 6
	EC_{50}	0.33	0.29	0.71	1.3	4.5	–	6.8	–	11.58	10.98	–	13.61	19.84	111.7
	R^2	0.96	0.96	0.99	0.97	0.96	0.86	0.96	0.94	0.73	0.9	0.97	0.95	0.94	0.83
DR-HepG2-Luc	RR_{AGmax}	48 ± 6	50 ± 7	44 ± 6	25 ± 7	43 ± 6	29 ± 2	21 ± 4	6 ± 2	15 ± 12	9 ± 2	7 ± 5	19 ± 8	8 ± 2	6 ± 2
	EC_{50}	1.6	2	2.6	7.05	2.08	2.6	28	13.9	–	–	17.75	–	11.32	–
	R^2	0.96	0.96	0.96	0.85	0.94	0.94	0.9	0.63	0.55	0.71	0.55	0.76	0.7	0.68
DR-T47D-Luc	RR_{AGmax}	133 ± 16	128 ± 3	109 ± 14	91 ± 15	120 ± 34	97 ± 10	31 ± 6	31 ± 4	39 ± 17	26 ± 8	31 ± 12	34 ± 6	27 ± 5	21 ± 8
	EC_{50}	5.6	10.7	10.8	–	–	5.3	9.6	31.85	17.28	25.34	17.58	10.28	10.29	24.26
	R^2	0.97	0.99	0.97	0.96	0.91	0.95	0.91	0.95	0.76	0.89	0.84	0.93	0.93	0.79
VM7Luc4E2	RR_{AGmax}	67 ± 11	54 ± 11	41 ± 8	49 ± 5	62 ± 10	20 ± 5	1 ± 1	88 ± 5	89 ± 5	31 ± 10	94 ± 5	29 ± 4	27 ± 7	7 ± 3
	EC_{50}	4.6	2.8	–	10.8	12.04	4.7	–	4.99	3.16	33.58	2.97	9.68	44.75	–
	R^2	0.93	0.94	0.93	0.98	0.97	0.75	–	0.98	0.95	0.82	0.97	0.93	0.84	0.72
AR-EcoScreen	RR_{AGmax}	26 ± 7	25 ± 7	31 ± 4	20 ± 8	37 ± 7	8 ± 3	12 ± 3	10 ± 1	12 ± 4	9 ± 2	16 ± 4	17 ± 7	13 ± 5	6 ± 2
	EC_{50}	2.2	1.5	8.1	2	–	–	–	–	–	–	–	–	–	–
	R^2	0.8	0.83	0.87	0.69	0.9	–	–	–	–	–	–	–	–	–
		Hexane extract - Antagonistic tests							Acetone extract - Antagonistic tests						
DR-EcoScreen	RR_{ANmax}	51 ± 22	27 ± 18	61 ± 11	47 ± 12	75 ± 15	50 ± 9	49 ± 17	59 ± 5	72 ± 27	34 ± 8	13 ± 5	43 ± 16	29 ± 16	23 ± 21
	IC_{50}	–	–	0.49	1.3	0.61	1.2	0.701	–	–	–	–	–	–	–
	R^2	–	–	0.77	0.68	0.85	0.66	0.75	–	–	–	–	–	–	–
DR-CALUX	RR_{ANmax}	116 ± 16	134 ± 28	72 ± 30	35 ± 1	134 ± 8	52 ± 18	20 ± 16	11 ± 9	12 ± 14	5 ± 4	7 ± 7	17 ± 12	6 ± 6	2 ± 10
	IC_{50}	1.4	1.3	4.4	–	2.4	–	0.88	–	–	–	–	–	–	–
	R^2	0.91	0.93	0.58	–	0.91	–	0.82	–	–	–	–	–	–	–
DR-HepG2-Luc	RR_{ANmax}	-22 ± 7	-36 ± 6	-29 ± 16	-55 ± 8	-42 ± 9	-46 ± 15	-4 ± 2	-23 ± 13	-19 ± 5	-30 ± 10	-40 ± 2	-24 ± 14	-33 ± 9	-32 ± 12
	EC_{50}	–	0.28	–	1.33	–	0.95	–	–	–	–	–	–	–	–
	R^2	–	0.64	–	0.74	–	0.64	–	–	–	–	–	–	–	–
DR-T47D-Luc	RR_{ANmax}	75 ± 23	72 ± 6	52 ± 28	-1 ± 14	44 ± 18	23 ± 20	-14 ± 14	2 ± 10	-1 ± 19	-7 ± 6	8 ± 2	-8 ± 17	-6 ± 9	5 ± 24
	IC_{50}	5.16	5.67	–	–	–	–	–	–	–	–	–	–	–	–
	R^2	0.86	0.89	0.69	–	0.73	–	–	–	–	–	–	–	–	–
VM7Luc4E2	RR_{ANmax}	-17 ± 1	-24 ± 10	-22 ± 1	-36 ± 1	-21 ± 12	-7 ± 24	-32 ± 7	2 ± 12	2 ± 16	9 ± 15	-8 ± 12	-11 ± 8	2 ± 2	-16 ± 6
	IC_{50}	0.33	0.4	0.47	0.44	0.36	–	–	–	–	–	–	–	–	–
	R^2	0.89	0.65	0.9	0.82	0.78	–	–	–	–	–	–	–	–	–
AR-EcoScreen	RR_{ANmax}	-38 ± 9	-40 ± 6	-2 ± 8	19	27 ± 8	-1 ± 21	67 ± 22	-17 ± 16	-13 ± 20	5 ± 23	-7 ± 23	15 ± 10	12 ± 29	23 ± 4
	IC_{50}	9.07	5.7	–	–	–	–	5.07	–	–	–	–	–	–	–
	R^2	0.69	0.81	–	–	–	–	0.71	–	–	–	–	–	–	–

F: fluff, D: dust, S: sludge, CT: control soil. Maximum relative responses (%) (RR_{AGmax} and RR_{ANmax}) ± SE (standard error) within the tested concentrations and half-maximum effect concentration (ng sample/ml cell medium) (EC_{50} or IC_{50}) for agonistic and antagonistic/co-exposure testes, respectively. R^2 is the coefficient of determination of the dose response curves. -: no response.

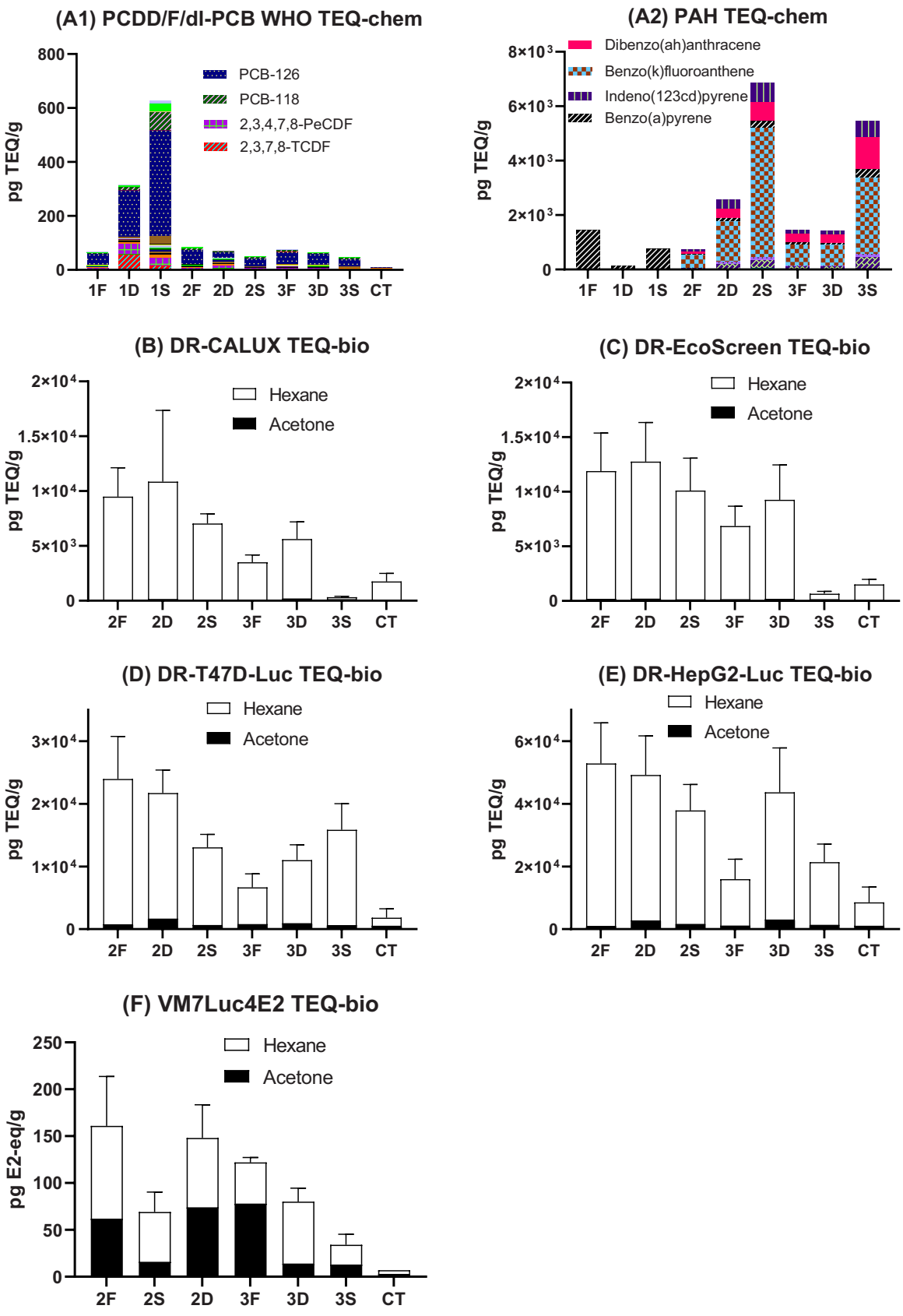

Fig. 2. Toxic equivalency (TEQ-chem) (pg TEQ/g) from chemical compositions according to the Toxicity Equivalency Factors (TEFs) of (Van den Berg et al., 2006) (A1) and Machala et al. (2001) (A2). Bioanalytical equivalents (TEQ-bio) (pg TEQ/g for dioxin-like activities (B–E) and E2-eq pg/g for estrogenic-like activities (F)) calculated from different bioassays.

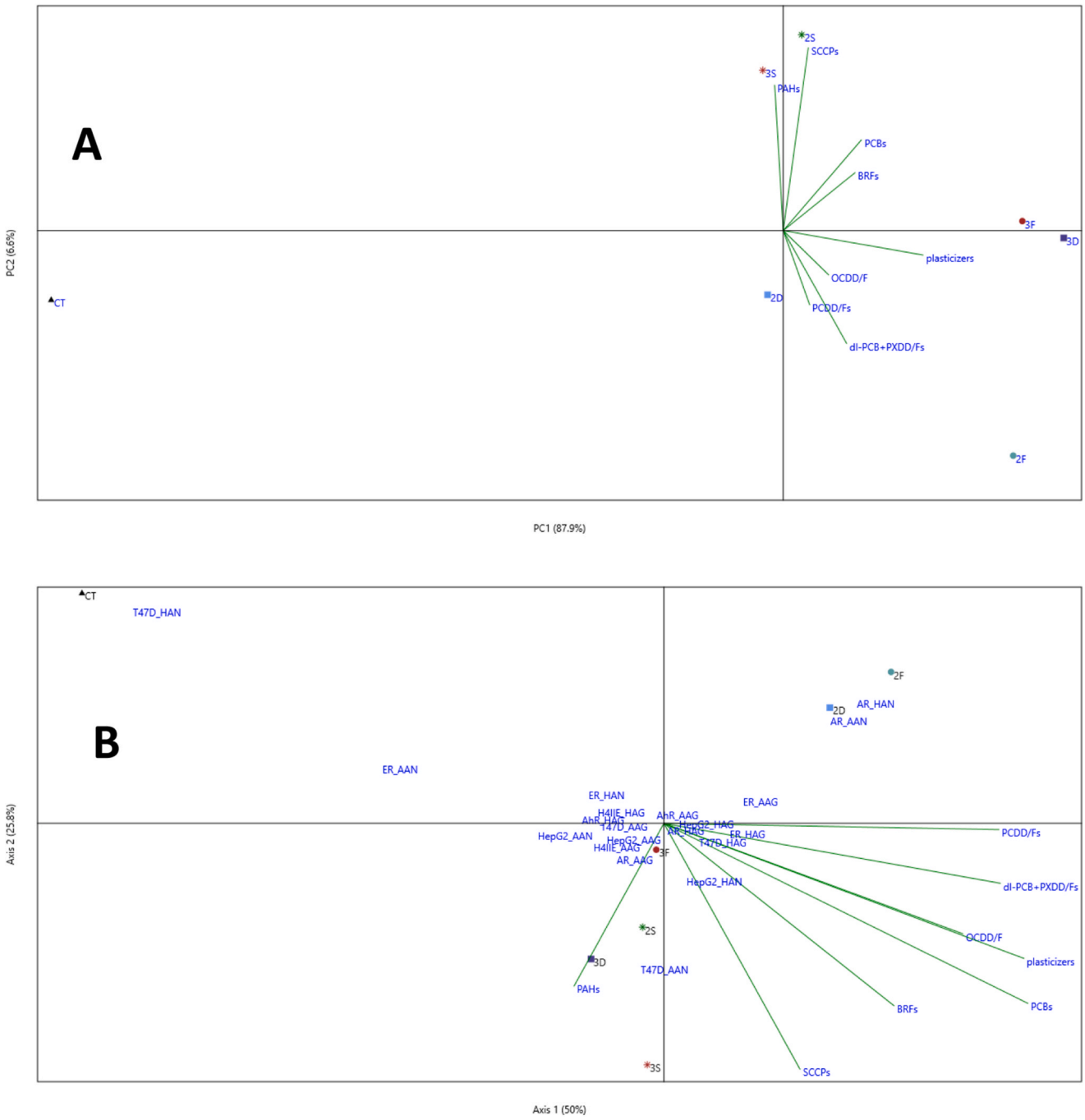

Fig. 3. Ordinations by principal components analysis (PCA) (A) and canonical correspondence analysis (CCA) (B) of fluff (F), dust (D), and sludge (S) samples collected from the 2 different shredding plants (sites 2 and 3). Detail input data of the PCA and CCA are presented in Table S2. HAG: agonistic tests for hexane extracts; HAN: antagonistic tests for hexane extracts; AAG: agonistic tests for acetone extracts; AAN: antagonistic tests for acetone extracts; AhR: DR-EcoScreen; H4IIE: DR-CALUX; T47D: DR-T47D-Luc; HepG2: DR-HepG2-Luc; ER: VM7Luc4E2; AR: AR-EcoScreen.

the full data set of chemicals (Fig. S6), indicating the validity of the 8 chemical variables to describe chemical characteristics of the samples. The 2 PCs were able to explain 87.9% and 6.6% of the variance. It is clear that most of the chemicals were grouped according to their chemical classification. While PAHs and SCCPs contributed most for PC2, the others weighted most for PC1. CT was out of the range of the diagram. Sludge samples 2S and 3S were grouped together due to their high content of PAHs, while samples 3F and 3D were close to each other along the PC1 due to their correlations with several groups. Samples 2D and 2F were plotted within the same quadrant, affected by mainly dioxins and dl-compounds (Fig. 3A).

The 8 (explanatory) chemical variables were used to explain optimally the biological variables (responses) of the bioassays by performing CCA using multivariate linear regressions. For the antagonistic tests, additive responses (positive values) which were already presented in the agonistic tests, were excluded from the correlation, while the negative values were converted into positive to ensure the scale of the correlation. Detail input data of the CCA are presented in Table S2. Fig. 3B shows the distributions of the bioassays and the samples respectively to the chemical variables. Axis 1 and 2 had the eigenvalues of 50% and 25.8%. None of the chemical and biological variables were correlated with CT, except antagonistic responses obtained for the hexane extracts on DR-

T47D-Luc (T47D_HAN). While the bioassays were not completely explained by the chemical variables, most of the assays were plotted along the PAHs, especially several acetone extracts listed as HepG2_AAN/AAG, H4IIE_AAG, T47D_AAG, and AR_AAG, highlighting the major contribution of this group on the bioactivities of the samples, especially 3D, 3F, and 2S. Meanwhile, several hexane extracts showed closer correlations with the groups of dioxins, BFRs, and PCBs with _HAG of ER, T47D, HepG2, H4IIE strongly correlated with PCDD/Fs and dl-PCBs+PXDD/Fs (Fig. 3B). Samples 2D and 2F were plotted closed to the AR antagonistic tests (AR_HAN and AR_AAN) as they showed distinct anti-androgenic responses for both hexane and acetone extracts (Table 3), while negatively related to PAHs. Their close cluster would also reflect their similar dl-activities and estrogenic activities (detail in 3.2). Samples 3D and 3F were close to the PAHs, indicating the important roles of PAHs to their bioactivities (Fig. 3B). The sludge sample of site 3 (3S) which displayed low activities in most of the assays (Table 3), regardless a similar chemical profile with 2S (Fig. 3A), was placed away from the others (Fig. 3B). The PCA and CCA ordinations indicated that the chemical profiles based on concentrations could not explain totally the biological responses of the samples.

4. Discussion

Several higher orders of magnitude in the mean concentrations of the contaminants in the samples from shredding plants than from the control site suggest that their source is the metal waste processed in the plants. The differences in POP contents among the 3 sampling sites (chlorinated and brominated compounds) and among the 3 sample types (PAHs, fluff < dust < sludge) reflect that the contamination sources are the waste being processed and the waste treatment methods used at these facilities, respectively. It is possible that due to longer time operation (~100 years earlier), site 1 was the first in the contamination levels of chlorinated compounds, especially in its sludge waste (1S) with at least 3 folds higher than the others (7.5 ng/g total PCDD/Fs and 84.5 μg/g estimated total PCBs (Table 2, Fig. 1A–C)). Site 3 that implements a 7000-horsepower shredder, led the BFR levels in dust with 53.4 μg/g (3D) compared to 1 μg/g in 2D (Table 2, Fig. 1D). One explanation can be that PBDEs and HBCD do not form chemical bounds with the flame-retarded materials. Thus, they can be released easily into the environment (dust) during waste treatment (possibly linked to high power shredder) (Finland MoE, 2016). The sludge samples displayed the highest PAH contaminations, which indicates high PAH contamination potential of wet shredders. The data for depositional fluxes (marked as DF, Table 2) (Dufour et al., 2020) which showed that site 2 was the most contaminated site, did not indicate any trend in the contamination profiles of our samples.

Literature chemical data for dust and sludge from shredder waste were very limited. The concentration of total PCDD/Fs in floor dust in our study is 20 folds lower than that recorded by Ma et al. for the e-waste recycling facilities in Eastern China (~2 ng/g compared to 40 ng/g (Ma et al., 2008)). They had also a higher figure for total PBDEs at 30 μg/g (Ma et al., 2009) but similar to our maximum of 51 μg/g for dust sample 3D (Table 2). However, their higher concentration of PCDD/Fs and PBDEs could be due to the formation of multiple chlorinated and brominated compounds during the open burning at low temperatures associated with the low-tech electronic waste recycling operations (Ma et al., 2009, 2008; Nguyen et al., 2019).

Meanwhile, most studies focus on (automotive) shredder residues ((A)SRs) which was the fluff in our study. The concentrations of PCDD/Fs were higher in this study than in others, while dl-PCBs and the TEQs were similar or smaller (Table 4). PCBs, PBDEs, and PAHs were within the same range or smaller in this study compared to the literature. Thus, the POP contamination profile of the 2019 fluff samples in this study seems to fit reasonably well with the usual ranges of the 30-year studied (A)SRs.

The study found phenanthrene, fluoranthene, and pyrene as the most

Table 4
Comparisons of contamination profiles of (automotive) shredder residuals ((A)SRs) in our study (fluff) and in the literatures.

References	Present study (fluff)		Sakai et al. (2014)	Cheminfo Services (2014)	Sakai et al. (1998)	Redin et al. (2001)		Yamamoto et al. (2007)	Osada et al. (2008)	Van Caneghem et al. (2010)	Mancini et al. (2010)	Santini et al. (2011)	Van Caneghem et al. (2012)	Arp et al. (2020)
Compounds	ASR (site 1)	SR (sites 2 and 3)	ASR	ASR[b]	ASR	ASR	SR	ASR	ASR	ASR	SR	ASR	ASR	SR
PCDD/Fs (pg TEQ/g)	16.8	18 ± 2	2.52–380	–	4.3–380	< 600	< 600	43–380	–	242–329	3.02–7	3.0–4	9000	–
dl-PCBs (pg TEQ/g)	49.4	62 ± 4	14–27	–	14–27	–	–	14–15	–	481–631[c]	–	–	–	–
PCDD/Fs (pg/g)	2423	2192 ± 872	–	150	970	–	–	–	970	–	–	–	–	–
dl-PCBs (ng/g)	321	384 ± 61	–	0.03	30	–	–	–	30	–	–	–	–	–
PCBs (ng/g)	2061[a]	2814 ± 33[a]	1.7–78510	5000	44–270	1100–6700	12,000	44–230	–	13,000–15000[d]	7930–24300	80.7–109.4	–	1800 ± 1400[e]
PBDEs (μg/g)	9.5	4.1	110–310	160	–	–	–	110–240	310	–	–	–	–	–
PAHs (μg /g)	–	12. ± 5.9	–	10	–	–	–	34–36	–	37–140	5.6–12	4–6.4	–	–

-: Not determined/Not mentioned.
[a] Sum of PCB-28 + PCB-31, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, and 12 dl-PCBs (Table S1)
[b] Asssumed concentration in ASR.
[c] Sum of PCB-77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, and PCB-189.
[d] Sum of PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180.
[e] Sum of PCB-28 + PCB-31, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-118.

dominant PAHs, which is in agreement with the results of Van Caneghem and Vandecasteele (2014) for ASR incinerators. We also showed the major contributions of the high molecular weight congeners OCDD and BDE-209 (Figs. 1A and 1D). That likely results from their high persistency and the most widespread use of decaBDE-209 (Finland MoE, 2016). This trend has been seen in several e-waste recycling sites (Hearn et al., 2012; Leung et al., 2007; McGrath et al., 2017). Also, several-fold higher concentrations for the 6 non dl-PCBs and PCB-118 used as technical PCB mixtures under different trade names (e.g. Aroclor and Clophen) compared with the other PCBs could be due to their historical applications (Fig. 1B-C). They were also found at high concentrations in e-waste and fine vehicle fluff in Norway (Arp et al., 2020). Another interesting finding is the similar profile of the non-dl PCBs from site 1 that have managed ELVs over 100 years with the Norwegian 2014 samples (e.g. coarse vehicle fluff) with high contributions of low chlorinated PCB-28 and PCB-52 (Arp et al., 2020) (Fig. 1C). Besides, different PCBs displayed similar proportions to the total PCBs of samples from sites 2 and 3 that manage both ELVs and e-waste with 30–50% contribution from PCB-28 + PCB-31 (Fig. 1B-C). The high content of low chlorinated PCBs in our samples could due to the environmental dechlorination of PCBs (Häggblom and Bossert, 2003; Rodenburg et al., 2012).

POP contamination seems to be a mixture of environmental background of an industrial country and the specific contaminants coming from the shredding facilities, whereas their persistence is a major problem. The high PCB levels and similar PCB profiles may indicate long-term/background contaminations of PCBs from the historical technical PCB mixtures via managing old vehicles in these facilities, especially for site 1. The 3 sites could also process old railway equipment together with a mixture of metal waste from various (unknown) origins, e.g. nonferrous metals, scraps from demolition works. Long-lived electrical equipment and the storage of obsolete equipment may further delay recycling and disposal of PCB contaminated e-waste, i.e. in sites 2 and 3 (Arp et al., 2020). Indeed, white goods have been found as the major PCB contributor in shredder waste back to the 20th century (Löfvenius et al., 2000). Precursor formation of PCDD/Fs and PCBs in metal waste can be seen as the by-products of chlorine-containing production or combustion processes (Kulkarni et al., 2008). For instance, high PCB levels were found in fuel and disposal fractions originating from ELVs (Löfvenius et al., 2000). Although none thermal process which can trigger the formation of PCDD/Fs and PCBs, is applied in the shredder plants, the metal fragmentation releasing heat (can reach to 70 °C) and small particles with higher surface/volume ratio, creates a highly favorable condition for the emission and the subsequent transfer of these semi-volatile chemicals into the studied residues (Dufour et al., 2020). Explosions and fires occurring accidentally in shredder facilities would create high temperature conditions favoring the formations of these chlorinated compounds.

In vitro characterization of the toxicity of metal shredder waste is very scarce with only few publications on the dioxin-like (dl-)activity of environmental samples from e-waste recycling sites in developing countries (Nguyen et al., 2013). Dl-activities observed for all hexane extracts (including CT), and to a lesser extent for the acetone extracts, indicate the dominance of dl-compounds in the extracts (Figs. S3 and S4). Different from AhR, for which ligands are typical highly lipophilic POPs (Avilla et al., 2020), ER has a more diverse group of ligands (Farooq, 2015). Hence, both hexane and acetone extracts were able to induce estrogenic-like activities (Figs. S5A and S5C, and Table 3). For example, bisphenol A, fluoranthene, and BaP were shown as ER agonists in our previous in vitro study (Doan et al., 2020), while both PCBs and PBDEs were considered as estrogenic-like compounds in yeast systems (Roszko et al., 2018). SCCPs exert potential estrogenic activities via the ERα in vitro (Zhang et al., 2016). Dioxins (e.g. TCDD) also induce estrogen-like activity which has been shown to involve the ER (Boverhof et al., 2006).

The activities of PAHs may show dominancy in AhR agonistic effects due to their high concentration in the samples. Even the AhR agonisms induced by the control soil could be explained by PAHs, considering that we did not quantify them in the sample. However, neither the response patterns of TEQ-bio follow the pattern predicted by PAH TEQs, especially for 3S (Fig. 2), nor the chemical and bioactivity profile can explain the distributions of all extracts in CCA ordination (Fig. 3B).

On the one hand, the cell responses for PAHs are cell-specific and depend on several exposure factors. For example, compared to the rat hepatoma DR-CALUX (EC_{50} BaP = around 10 μM, RR_{AGmax} = 171%), the human hepatoma DR-HepG2 responds lower with EC_{50} = 16.8 μM, RR_{AGmax} = 27% (Doan et al., 2020). Due to the high metabolic clearance rate of PAHs, especially in liver cells, time of exposure can also affect the result. We used 24 h of exposure instead of 4 h (Pieterse et al., 2013) when the maximum response for PAHs is obtained, since we targeted on the mixed samples as a whole and more on the persistent response of the POPs. Meanwhile, the detoxifying/toxifying action of PAHs can also lead to the generation of highly genotoxic metabolites (Höner, 2001).

On the other hand, the bioactivities are rather a more complex combined effect of mixtures of chemicals in the samples including unknown chemicals interacting with one another towards different cell lines specifically. The murine cells demonstrated higher sensitivity and stronger responses towards dioxin-induced AhR transactivation than the human cells (Doan et al., 2019). That could be the reason why we could not observe any AhR antagonism in the 2 murine cell lines. Nevertheless, this study showed a higher sensitivity of the human hepatoma DR-HepG2-Luc towards POP-induced AhR antagonisms than the 2 murine cell lines. Several dominant PCBs including PCB-128, PCB-118, and PCB-138 and BDEs including BDE-47 were AhR antagonists (Doan et al., 2019), thus probably explaining the AhR antagonist effects in DR-HepG2-Luc (Fig. S4B and Table 3). Similarly, within PAHs, fluoranthene and phenanthrene were pure AhR antagonists for both human and murine cells. Meanwhile, BaP was a potent AhR agonist, especially for the human mammary gland DR-T47D-Luc, which could refer to the high AhR agonistic activities of the extracts on this cell line (Fig. S4E) (Doan et al., 2020).

In this study, TEQ-chem calculated from chemical analyses were several folds lower than TEQs from bioassays (TEQ-bio) for compounds mediating the AhR agonisms. That, first, may be due to some missing data of the PAH TEFs (Machala et al., 2001). However, the lack of TEF values for other dl-compounds such as PBDD/Fs or PXDD/Fs may also underestimate the TEQ-chem calculation (Nguyen et al., 2019, 2013). In soils and dusts from Asian informal e-waste recycling sites, the concentrations of PBDD/Fs were comparable to or higher than those of PCDD/Fs by up to one order of magnitude (Nguyen et al., 2013). For example, floor dust in e-waste recycling facilities in Fengjiang, China contained PBDD/Fs in the range of 89.6–143 ng/g (2,3,7,8-substituted congeners only) (Ma et al., 2009). PBDF TEQs (7.9–5400 pg/g dry weight) were similar or higher compared with PCDD/F TEQs (6.8–5200 pg/g dry weight), whereas PXDFs were also significant TEQ contributors in open burning areas in Agbogbloshie e-waste site, Ghana (Nguyen et al., 2019). Besides, heavy metals are also the main sources of contamination in shredder waste (Frazzoli et al., 2010). Although we aimed to extract organic pollutants, several lipophilic heavy metals can also be extracted which can affect the bioactivities. For example, mercury has been found in metal scraps coming from both ELVs and e-waste (Houessionon et al., 2021; Sastry et al., 2002). While several lipophilic mercury compounds e.g. methylmercury have been detected in municipal solid waste (Cheng and Hu, 2012), their presence in shredder waste is deserved further investigation considering their highly toxic and bioaccumulative profile. To some extent, TEQ-bio, especially ones based on human cell lines, would reflect more precisely the human health concerns of the adverse effects of sample mixtures rather than TEQ-chem, which normally cannot identify the presence of all compounds in the mixtures.

This study also raised the concern of potential adverse effects of POPs contaminating shredder waste on human health. Working or living in an

environment contaminated with POPs results in the exposure to those chemicals via inhalation and dermal absorption, soil ingestion, consumption of food cultivated in the contaminated areas, etc. Table 5 shows the estimated daily exposures of workers to dl-compounds working in the shredder facilities based on the model proposed by Nouwen et al. (2001) and simplified by Nguyen et al. (2003) (Eqs. (6) and (7)). Daily intakes of dl-compounds were estimated through ingestion and dermal absorption of dust and soil (fluff) according to both PCDD/F/dl-PCB WHO TEQ-chem and DR-HepG2-Luc TEQ-bio for an adult of 70 kg body weight (Wt).

$$\text{Soil/dust ingestion: } DU_{total}=DU_{soil}+DU_{dust}=(AID\times IF_{soil}\times C_{soil})/Wt+(AID\times IF_{dust}\times C_{dust})/Wt \quad (6)$$

$$\text{Dermal exposure: } DA_{total}=DA_{soil}+DA_{dust}=(DAE_{out}\times EF_{out}\times C_{soil})/Wt+(DAE_{in}\times EF_{in}\times C_{dust})/Wt \quad (7)$$

DU/DA_{total}, DU/DA_{soil} and DU/DA_{dust}: total ingestion/dermal exposure of dl-compounds from soil and dust, respectively (pg TEQ/kg bw/day); AID: ingestion of soil/dust particles (2.6×10^{-5} for soil/dust or 0.02 for soil and 0.03 for dust according to US EPA soil/dust ingestion (2011), kg/day); IF_{soil} and IF_{dust}: ingestion factor for soil (0.237) and dust (0.55); C_{soil} and C_{dust} (pg TEQ/g): concentrations of dl-compounds in soil and dust; DAE_{out}/DAE_{in}: skin coverage with dust outside (0.0375 kg/m^2) and inside (0.00056 kg/m^2); EF_{out}/EF_{out}: exposure factors for appropriate absorptions of outside (0.0011) and inside (0.0131) skin.

Table 5
Estimated daily intakes (pg TEQ/kg bw/day) of PCDD/F/dl-PCB WHO TEQ-chem and DR-HepG2-Luc TEQ-bio for workers working in the shredder plants via soil (fluff)/dust ingestion and dermal exposure, compared with daily intakes of PCDD/Fs from study sites in Belgium, China and Southern Asian counties.

Studies		Soil + dust ingestion	Dermal exposure	Total daily uptake	Soil + dust ingestion*
Metal shredder plants, Wallonia, Belgium[a] (PCDD/F/dl-PCB WHO TEQ-chem)	site 1	0.07	0.072	0.142	78.71
	site 2	0.022	0.057	0.079	21.95
	site 3	0.02	0.052	0.072	20.51
Metal shredder plants, Wallonia, Belgium[a] (DR-HepG2-Luc TEQ-bio)	site 2	14.7	36.3	51	15,175
	site 3	10.32	13.96	24.28	11,374
Municipal waste incinerators, Wilrijk, Belgium[b]		0.0079	0.0073	0.0152	–
Dumping sites, Hanoi, Vietnam[c]		0.03	0.071	0.101	–
Dumping sites, Philippines[c]		0.16	0.38	0.54	–
Dumping sites, Cambodia[c]		0.12	0.28	0.4	–
Dumping sites, India[c]		0.02	0.04	0.06	–
E-waste recycling base, Taizhou, China[d]		0.22	0.14	0.36	–

-: Not determined.
* Estimated based on recommended US EPA soil/dust ingestion.
[a] This study.
[b] Data cited from Nouwen et al. (2001).
[c] Data cited from Nguyen et al. (2003).
[d] Data cited from Ma et al. (2008).

The highest daily uptake of dl-compounds was from site 1 with 0.142 pg WHO TEQ/kg bw/day, followed by sites 2 and 3 with 0.079 and 0.072 pg TEQ/kg bw/day, respectively. Using the US EPA (2011) recommended values for daily soil + dust ingestion for adults, the figures were 3 orders of magnitude higher due to the high AID. The data from DR-HepG2-Luc TEQ-bio were highest and the first of its kind, about 500 times higher than those from WHO TEQ-chem (Table 5).

In fact, food consumption contributes for over 90% of human exposure to dioxins and dl-PCBs (WHO, 2010), and EFSA recommends a tolerable weekly intake from food of 2 pg TEQ/kg bw/week (EFSA, 2018) (corresponding to 0.29 pg TEQ/kg bw/day). However, this value strongly underestimates the carcinogenic potential of dioxins. In the consolidated table of Office of Environmental Health Hazard Assessment (OEHHA)/Air Resources Board (ARB) approved risk assessment health values (State of California, October 2, 2020) (OEHHA/CARB, 2020), the oral slope factor for TCDD (1.3E+05 mg/kg bw/day) corresponds to a one in a million excess risk for an ingestion of 7.7 fg/kg bw/day. This latter value is 38 times lower than the EFSA recommendation. The high exposure of shredder workers to dl-compounds as estimated in Table 5, likely increases their body burden of dl-compounds (TEQ-chem based), or even potentially causes adverse health effects as this exposure is several folds higher than the recommended tolerable daily intake (TEQ-bio based).

For comparison, shredder workers show a 5- to 10-fold higher estimated ingestion and dermal absorption of dl-compounds (TEQ-chem based) via soil and dust from the shredder facilities than ones living in the vicinity of municipal waste incinerators (Nouwen et al., 2001) in Belgium also. However, these figures were 3 to 7 folds lower compared to e-waste recycling base in China (Ma et al., 2008) or to the dumping sites in Philippines and Cambodia, and similar to the dumping sites in Vietnam and India (Nguyen et al., 2003) (Table 5).

The high levels of POPs in human and food samples from the surrounding areas of metal shredder plants and e-waste recycling sites have been documented widely (Eguchi et al., 2015; Hoang et al., 2020; Labunska et al., 2013; Tao et al., 2016). That indicates the potential of POP releases into the environment during treatment processes of metal waste. The concern of potential adverse effects on human and environmental health has been raised in the previous study on these 3 metal shredder plants (Dufour et al., 2020). Indeed, elevated concentrations of PCBs were detected in vegetable, farm animal products, and human hairs from the surrounding areas. Besides, by applying modeling theoretical exposure scenarios, Wallonia has acknowledged a moderate risk or chronic toxicity associated with the shredder plants. This will be checked in the near future through a blood/urine biomonitoring in the exposed population.

5. Conclusion

This study revealed the occurrences and adverse effects of toxic organic pollutants in fluff, dust, and sludge samples collected from 3 shredder plants located in Wallonia, Belgium, 2019. While the concurrence of chlorinated and brominated compounds was site-specific, the PAH content was dependent on types of samples. We showed the high contamination levels of not only fluff ((A)SRs), but also of dust and sludge samples. Especially, high concentrations of PCDD/Fs and PCBs were found in sludge sample from site 1 (1S), of BRFs in dust sample from site 3 (3D), and of PAHs in sludge samples from sites 2 and 3 (2S and 3S). The samples induced significant dioxin- and estrogenic-like activities, which were rather a combined effect of mixtures of chemicals. By combining chemical and biological analyses, we were able to reveal the toxicity of mixtures of toxic organic pollutants in shredder residues and gave suggestions for their risk assessment. High estimated ingestion and dermal absorption of dl-compounds in shredder workers and high levels of contaminants in human and food sample from the

surrounding areas of metal shredders, raise important concerns.

CRediT authorship contribution statement

Doan TQ: Conceptualization, Methodology, Investigation, Writing – original draft. **Pham AD**: R software performance, Writing – review & editing. **Scippo ml**: Writing – review & editing. **Brouhon JM**: Writing – review & editing. **Lundqvist J**: Writing – review & editing.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.127009.

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