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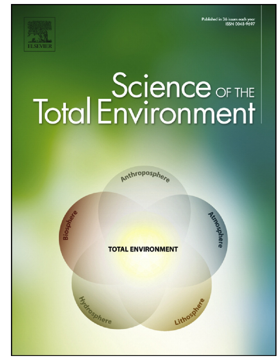

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A.R. Fernandes, I.R. Lake, A. Dowding, M. Rose, N.R. Jones, R. Petch, F. Smith, S. Panton

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The Potential of Recycled Materials used in Agriculture to Contaminate Food through Uptake by Livestock

[1,3]A. R. Fernandes*, [1]I. R. Lake, [2]A. Dowding, [3,4]M. Rose, [1]N. R. Jones, [3]R. Petch, [3]F. Smith, [3]S Panton,

[1]School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK
[2] Food Standards Agency, Clive House, 70 Petty France, London, SW1H 9EX.
[3]Fera Science Ltd, Sand Hutton, York, YO41 1LZ, UK.
[4] Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN, UK

Author for correspondence: A. R. Fernandes
E-mail: Alwyn.Fernandes@uea.ac.uk

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Abstract

The potential for contaminant uptake from recycled materials used in livestock farming, to animal tissues and organs, was investigated in three practical modular studies involving broiler chickens, laying chickens and pigs. Six types of commercially available recycled materials were used either as bedding material for chickens or as fertilizer for cropland that later housed outdoor reared pigs. The contaminants studied included regulated contaminants e.g. polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs, dioxins) and polychlorinated biphenyls (PCBs), but related contaminants such as polybrominated diphenylethers (PBDEs), hexabrominated cyclododecane (HBCDD), polychlorinated naphthalenes (PCNs), polybrominated dioxins (PBDD/Fs) and perfluoroalkyl substances (PFAS) were also investigated. Contaminant occurrence in the recycled materials was verified prior to the studies and the relationship to tissue and egg concentrations in market ready animals was investigated using a weights of evidence approach. Contaminant uptake to animal tissues and eggs was observed in all the studies but the extent varied depending on the species and the recycled material. PCBs, PBDEs, PCDD/Fs, PCNs and PFAS showed the highest potential to transfer, with laying chickens showing the most pronounced effects. PBDD/Fs showed low concentrations in the recycled materials, making it difficult to evaluate potential transfer. Higher resulting occurrence levels in laying chickens relative to broilers suggests that period of contact with the materials may influence the extent of uptake in chickens. Bio-transfer factors (BTFs) estimated for PCDD/F and PCBs showed a greater magnitude for chicken muscle tissue relative to pigs with the highest values observed for PCBs in laying chickens. There were no significant differences between BTFs for the different chicken tissues which contrasted with the high BTF values for pigs liver relative to muscle. The study raises further questions which require investigation such as the effects of

repeated or yearly application of recycled materials as fertilizers, and the batch homogeneity/consistency of available recycled materials.

1. Introduction

In recent decades, dwindling trends and increasing costs of natural resources have driven a requirement for sustainable practices and more efficient utilisation. A key outcome of this realisation has been an upturn in recycling, and an increasing number of materials that would previously have been disposed of as waste are now reused. This process was also encouraged by regulation in different parts of the world, e.g. regionally, as in the European Union (EU) landfill directive (European Commission, 1999), nationally in Japan with an itemised series of regulations (MOE, Japan 1998), or state-specific regulation as seen in the US and Canada. Recycling within the farming industry is not a new concept and historically, river sediments and municipal sewage sludge have been used to improve the fertility of cropland. In more recent times, the nutrient-rich organic matter that is derived from the treatment of domestic sewage, called biosolids, is viewed as a useful crop fertilizer (Deeks et al., 2013; Rigby et al., 2015). This use of biosolids is a well-established sewage sludge disposal method and the practice is regulated in the UK (Public health England and Wales, 1989; Department of the Environment, 1996) in order to control the levels of certain potentially toxic elements (PTEs) in soil following application. The regulations however, do not include other persistent and regulated organic contaminants such as polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs, dioxins), polychlorinated biphenyls (PCBs) dioxins, polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs). These food contaminants are known to occur at appreciable concentrations in biosolids along with other emerging contaminants such as brominated flame-retardants (BFRs), perfluoroalkyl substances (PFAS) and pharmaceuticals (Smith, 2009; Clarke and Smith, 2011; Rigby et al., 2015). This occurrence can be a real concern as evidenced by a recent contamination incident in Alabama in the United States (US) where a drinking water provisional health advisory (EPA, 2016) was issued after high PFAS concentrations levels in biosolids that were spread to agricultural land, resulted in the contamination of soil, groundwater, grass and beef.

More recently, a number of other recycled materials that originate from industrial use have become available, both to arable as well as livestock farming, either as fertilizers, soil conditioners or animal bedding. These include bedding material such as recycled wood, dried wastes from the paper industry – kiln-dried paper sludge (DPS) and shredded recycled cardboard, and fertilizer materials such as meat and bone meal ash (MBMA) and poultry litter ash (PLA) (Environment Agency, 2012), all of which were investigated in this study.

Untreated waste wood, such as pallets or packing material, is generally regarded as a safe and beneficial resource that can be recycled to generate wood shavings for use as animal bedding. It has previously been proposed that bedding made from 'visibly clean waste wood' could form an acceptable low risk alternative for the production of animal bedding material and current regulatory advice on the use of waste wood is available (Environment Agency, 2018). The accompanying economic benefits of this type of waste wood recycling would be the provision of affordable, comfortable and hygienic livestock bedding against a backdrop of rising costs and scarcity of traditional materials such as straw. These suggestions however, assume low levels of ingestion of bedding material by livestock; reliable traceability of waste wood; vigilant removal of visibly contaminated wood; and clear traceability when wood was treated with potentially harmful chemical. There is very little, if any information on livestock ingestion rates of bedding. Additionally, a large proportion of the more toxic contaminants such as PCDD/Fs, BFRs, and PCBs - would not be clearly visible. This has been illustrated by studies in the past (Firestone et al., 1979; Fries et al., 1999; Brambilla et al., 2009) that show raised PCDD/F levels in milk and eggs resulting from the ingestion of pentachlorophenol treated wood (commercial pentachlorophenol was widely used as a wood preservative and was later found to contain PCDD/Fs as impurities).

Waste paper, cardboard and dried paper sludge makes highly absorbent bedding that is light and mobile, with low moisture content (e.g. approximately 10% for waste paper/board) and low density. Waste cardboard can be used for a variety of livestock when it is shredded into small pieces. Dried paper sludge is also a commercially available material which is produced using the waste paper crumb by-product from the paper recycling industry. This product can be processed and dried to yield a pale grey friable material with good desiccant properties and approximately 95% dry matter. It also provides a highly absorbent and effective bedding product, with high absorbency, good thermal properties, produces little dust and degrades quickly. Additionally, it tends to have low spore and pathogen levels.

Ash products are derived from incineration and are generally used to improve soil quality and nutrients. Poultry litter ash (PLA), produced and supplied as a fertiliser for arable crops and grassland contains significant levels of phosphate and potash, Meat and bone meal ash (MBMA), may be produced from slaughterhouse waste containing a minimum of 80% animal tissue plus a maximum of 20% of waste sludge that arises from cleaning processes when food of animal origin is prepared. This feedstock is incinerated to produce ash that is relatively rich in phosphorus, a scarce mineral used as fertilizer for agricultural land. Newer production systems burn meal, producing ash that could be used as the raw material for phosphorus fertilizer. It has the added advantage of being low in cadmium compared with rock phosphate.

The drawback however, is that most types of ash, including PLA and MBMA can potentially contain high levels of contaminants such as PCDD/Fs and various PTEs (Rigby et al., 2015) if the combustion conditions and incinerator feedstocks are not carefully managed. In order to control the level of contamination in these materials, the Waste and Resources Action Programme (WRAP) and the UK Environment Agency have developed a Quality Protocol for PLA that sets limits for certain PTEs and for dioxins, e.g. 20 ng WHO-2005 TEQ/kg for individual batches of material produced (Environment Agency, 2012). A similar quality protocol is under development for MBMA, for which, PCDD/F concentrations of over 120 ng WHO-2005 TEQ/kg have been reported. Other similarly recycled materials such as paper sludge ash (PSA) have been found to contain relatively low PCDD/F concentrations (typically <4 ng WHO-2005 TEQ/kg) but there is evidence that some examples of PSA can contain higher PCDD/F concentrations.

These contaminant occurrences coupled with the increased use of various recycled materials and the potential for inadvertent assimilation by livestock (e.g. ingestion during feeding) have created a growing need for information about levels of ingestion of these different types of bedding under various conditions, the types and levels of contaminant present in these recycled bedding materials, and (to mimic the worst-case scenarios) the impact on contaminant levels in produce from livestock reared on contaminated bedding. The exposure to multiple contaminants through this route, with cumulative or competing effects could introduce another potential facet to this work. However, the additional requirements of mathematical modelling, quantitative structure-activity relationship models, and toxicological concern threshold data (Pose-Juan et al., 2016) were financially beyond the scope of the current study.

The primary objective of this study was to provide evidence on the occurrence of contaminants in recycled materials and investigate the potential for the contaminant transfer from these materials to livestock-derived food produced when they are used in agriculture. This would allow evaluation on whether the use of recycled materials in agricultural food production compromises food safety, whilst recognising the benefits in terms of sustainability and efficiency associated with the use of materials that would otherwise be classed as waste. The study broadly comprises two modules – the first on poultry was sub-divided into broiler chickens and egg production, while the second investigated outdoor pigs. The recycled materials have a different utility in these studies - for the poultry modules, the materials were used as bedding, whereas for the outdoor pig module the materials were used as fertilizers for the cropland on which the animals were reared. Although the extent of bioavailability would vary in these utilisations, the potential primary uptake pathway i.e. ingestion, would be the same. The study also provided an opportunity to investigate biotransformation for some contaminants. Biotransformation refers to the ability of the animals to enzymatically convert contaminants (mostly lipophilic) to more water-soluble derivatives. This process which is often partial and applies to all xenobiotics, proceeds through the well-defined metabolic processes of hydrolysis, reduction and oxidation of the primary contaminants. The resistance, or stability of contaminants such as PCDD/Fs and PCBs to these processes, is species dependent and has led to studies that attempt to quantify the relative extents to which contaminants are bio-transformed. Biotransformation should be considered as an indicative measure of transfer given uncertainties arising from day-to-day variations in contaminant input and genetic variability and to a smaller extent, measurement uncertainty.

The modules were designed to provide physical evidence that can be used to evaluate contaminant uptake by livestock and eventually, to allow evaluation of the adequacy of current or proposed quality protocols or other controls on the use of recycled materials for primary food production. Modern, established and contemporary farming methods were used to raise the animals, and so reflected typical agricultural practices (Foxall et al., 2004). It should be remembered, however, that these have an associated potential for allowing contaminant entry into the food chain. For example, chickens and eggs have been found to assimilate contaminants as a result of PCB/dioxins exposure from contaminated feed in earlier food safety incidents, e.g. the Belgian PCB/dioxins incident (Bernard et al., 1999).

2. Experimental

The study was based on the principle that animals would be raised in batches associated with different recycled materials along with a control (a material that was previously established to be uncontaminated, or no material in the case of the pigs), using conventional farming practices. At market readiness (defined by conventional farming practice), the uptake of contaminants to edible tissue or eggs was determined by chemical analysis and evaluated against a control.

2.1 Recycled materials

A number of recycled materials are in contemporary or recent use in the UK and a first step was to identify suitable materials for use in the study based on commercial availability and current husbandry practices. A listing of currently available candidate materials and selection considerations is given in the Supplementary information (SI) Section 2.1.

Four recycled materials were selected for the poultry farming modules. These were shavings from recycled wood, shredded recycled cardboard and dried paper sludge/pulp (DPS). Shavings from untreated, unused wood which were known to be "clean" from previous use were used as the control material. In conventional outdoor pig farming, recycled materials are used as soil enrichers or fertilizers for a crop which in this case was grass. After harvesting, the land is turned over for raising pigs. Three recycled materials were selected for the pig module - Poultry litter ash (PLA), Meat and bonemeal ash (MBMA) and biosolids. The control for the module was an untreated (no material) strip of land with a history of no recent chemical use.

The recycled materials were analysed (see section 2.4) as received from the suppliers after thorough homogenisation and particle size reduction (biosolids were first dried at 18 - 20ºC on a pre-cleaned foil lined tray to constant weight). The shredded cardboard and wood shavings were homogenised in a centrifugal mill.

2.2 Poultry Modules

All animals in both modules were housed within the same enclosure. The housing was divided into 4 quadrants, each used for the different bedding materials (recycled wood shavings, recycled cardboard, DPS and clean wood shavings) and separated by solid partitions. Conditions such as light, temperature and humidity were monitored and recorded. Water and feed were provided *ad libitum*, and the physical condition of the animals was monitored during the course of the studies. Samples of feed were collected and analysed as part of the study.

2.2.1 Broilers

Broilers were reared from identical chicks of the same strain. They were obtained as day-old chicks from a single source in order to minimise variability. Approximately 25 birds were raised within each quadrant of recycled material/control. The feed varied according to the growing cycle of the animals - starter feed from weeks 0 – 3, progressing to grower feed until market ready which in the case of this strain was 8 weeks. At this stage the birds were anesthetised, killed and butchered. Muscle tissue was taken from the breast and thighs, skin, fat and liver were collected for analysis. Care was taken to keep the animals from different treatments separate during sample collection. Chicks on the recycled cardboard material were placed on the control wood for the first two weeks, on the advice of the veterinarian who considered that the particle size of the material was too large for young chicks to maintain body temperature.

2.2.2 Laying chickens

Laying chickens are raised primarily for eggs, but are also used for meat when the rate of egg production becomes commercially unviable. The lay-out of the animal housing was essentially the same as for the broilers, and the same recycled materials were used in this module. As for the broilers, birds from the same strain were obtained as day-old chicks from a single source and sexed to provide hens for the study. The life-cycle for laying hens, including the productive egg-laying period is considerably longer than for broilers. Starter feed was provided from 0 - 6 weeks, followed by grower's feed until the end of the study. The time corresponding to the onset of egg laying (approximately 4 months) was recorded and eggs were collected at regular intervals as detailed later in the results section (Table 3.3). Eggs collected at these intervals were pooled for each material and after planned collection intervals, the birds were anesthetised, killed and butchered. Muscle tissue taken from the breast and thighs, skin, fat and liver were collected for analysis. Animal segregation between different treatments was maintained during sample collection.

2.3 Pig module

A contiguous portion of agricultural land that had not been recently used (in order to minimise the possibility of contaminant carryover from previous use) was identified and divided into four plots, each separated by a 10 m buffer strip (See Figure 1). One of these was designated as the control. PLA, MBMA, and biosolids were applied to each of the other three plots respectively in supplier specified amounts (MBMA and PLA 1 tonne per hectare and biosolids 25 tonne per hectare), using commercial spreading equipment (therefore following normal agricultural practice). Following application, the field was ploughed and seeded with grass. The crop was harvested when ready at 4 months from sowing. The plots were then fenced individually to separate each treatment with the introduction of an arc housing (see detail in Figure 1), and the entire area was fenced in order to prevent escape.

Although 12 animals were required per treatment, 19 piglets were used as this was closer to normal farming practice and to improve animal welfare through enhanced thermal mass during the winter. Practically, it also provided backup in case of animal mortality.

In order to reduce the effects of genetic variability, piglets were introduced in sets of 4 each from the same sow (i.e. 1 sibling into each plot) and were ear tagged for identity. The animals were not gender matched as there is no evidence that the growth period to market readiness (approx. 5 months from introduction to plot) influences gender-selective contaminant uptake. Weaner feed was provided for the first two weeks, then grower feed for the next two months followed by finisher feed to study-end. Supervision and veterinary care was provided during the study.

Following market readiness, the animals were identified with a skin mark, then slaughtered and butchered on the same day. The liver and 1.5 kg of muscle tissue from the right shoulder of each pig was taken for analysis. The samples were placed in individual labelled sampling boxes lined with pre-washed foil, then wrapped in the foil. Soil from the plots was also sampled, pre-study (to ensure that background contamination of the plots did not vary) and post-spreading of the materials. Soil was sampled by taking 7 sub-samples from each plot (to 5 cm depth), and homogenising and sieving (4 mm mesh) these during drying (18 - 20ºC) to form a composite.

2.4 Analysis

In addition to the tissue samples from each of the modules – muscle, (chicken) skin and liver, feed samples used in the study were also analysed after homogenisation and grinding, as were soils as described in 2.3 above. Eggs and tissue samples were homogenised before and after freeze-drying to yield stabilised sample aliquots for analysis, except for PFAS which was analysed using aliquots of fresh homogenised sample, pre-drying.

2.4.1 Analytes

The following analytes were determined: Regulated contaminants and EC-recommended PBDEs are highlighted in bold.

- **PCDD/Fs - all 17, 2378-Cl substituted PCDDs and PCDFs.**
- PCBs - IUPAC numbers: (dioxin-like) **77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189.** (non-dioxin-like) 18, **28**, 31, 47, 49, 51, **52**, 99, **101**, 128, **138, 153**, **180**.
- Brominated dioxins - 2,3,7-T_3BDD, 2,3,8- T_3BDF, 1,2,3,4,6,7,8-H_7BDF and nine, tetra to hexa- brominated PBDD/F congeners.
- PBDEs - IUPAC numbers 17, **28**, **47, 49**, 66, 71, 77, 85, **99, 100**, 119, 126, **138, 153, 154, 183 and 209.**
- PBB congeners: IUPAC numbers 15, 49, 52, 77, 101, 126, 169,153 and 209.
- PCNs - PCN-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, & 75
- α-HBCDD, β-HBCDD and γ- HBCDD
- PFAS - Perfluorooctanesulfonylamide (PFOSA), Perfluorobutane sulfonate (PFBSH), Perfluorohexane sulfonate (PFHxS), Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDeA), Perfluoroundecanoic acid (PFUnA) and Perfluorododecanoic acid (PFDoA).

Additionally, PTEs, TBBPA, mixed halogenated dioxins and biphenyls (PXDD/Fs and PXBs) were also analysed, but are not reported here.

2.4.2 Analytical Methodology

All of the analytical methodologies used in this study have been published before:

- PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PBBs, PXBs (Fernandes et al., 2004; 2008; 2011)

- PBDE and PCN analysis (Fernandes et al., 2004; 2010)
- HBCDD/TBBPA (Driffield et al., 2008)
- PFAS (Clarke et al., 2010)

The methodologies are all based on isotope dilution analysis using ^{13}C-labelled analogues of target analytes and high resolution mass spectrometric (GC-HRMS) or tandem mass spectrometric analysis (LC-MS/MS). The methodologies have been robustly validated and extensively used in other studies. More information along with details on quality aspects and references are given in the SI section 2.4.

2.5 Data analysis

The principle diagnostic output of this study was to establish, through comparison of tissue or egg concentrations against a control, whether any effects of the use of recycled materials were discernible. Although basic statistical outputs are used, the practical consideration of cost did not allow the volume of sample data that would support a purely statistical evaluation. Thus the basic statistics used here were reinforced using the weights of evidence approach (Weed, 2005; Linkov et al., 2009) in order to comparatively evaluate concentration data. This is a quantitative approach for combining evidence to support a hypothesis and was originally developed to support medical diagnosis. It is now used in a number of different fields including environmental food contaminants (Lake et al., 2014). The practical application of the approach in this work is demonstrated in Figure 2, where tissue concentrations, ordered from lowest (animal 1 tissue) to highest (e.g. animal 4 tissue) for a particular analyte in a control group (left column), are compared to the corresponding data for the recycled material treated group (right column). The strength of evidence can then be characterised and represented symbolically as follows:

- ↑ **Strong evidence of uptake** – the minimum concentration in the recycled material group exceeds the maximum concentration of the control group (Fig. 2A LHS)
- ↑ Some evidence of uptake – the minimum concentration in the recycled material group is greater than the median concentration of the control group. (Fig. 2B LHS)
- - no evidence of any uptake
- x concentration below LOQ, comparison not possible.

Where more specific comparison is possible, e.g. for matched siblings in the pig module, or for eggs taken at a particular time period, a similar, weight of evidence comparison can be made. However, in this case the comparator is the corresponding matched control sibling (or the corresponding control egg sample) as shown below, and visually in the boxes on the right in Figure 2. This symbolic notation was used in Tables 3.2 to 3.4.

- ↑ **Strong evidence of uptake** – when all individual concentrations in the recycled material group exceed the corresponding control concentrations (Fig. 2A RHS)
- ↑ Some evidence of uptake – when the majority of individual concentrations in the recycled material group are greater than the corresponding control concentrations. (Fig. 2B RHS)
- - no evidence of any uptake
- x concentration below LOQ, comparison not possible.

There is a final qualification for acceptance of an observation as "strong evidence", and this is simply a confirmation that in addition to comparison to the control, the contaminant must also be present in the recycled materials (or controls). This final stage allows for validation of the observation that are made.

2.6 Biotransfer factors (BTFs)

BTFs were estimated for PCDD/Fs and PCBs by dividing the contaminant concentration in a particular tissue or eggs (as ng/kg fat) by the daily contaminant input flux which was expressed in ng/day (Fries, 1999; Foxall et al., 2004; Fernandes et al., 2011B).

$$\text{Bio-transfer factor (BTF)} = \frac{\text{Tissue concentration (ng.kg}^{-1}\text{ fat)}}{\text{Daily contaminant input flux (ng.d}^{-1}\text{)}}$$

Ideally BTFs should be calculated during a steady state period during the growth cycle (e.g. when contaminant inputs and outputs are in equilibrium). However, it is debateable as to whether this state is realised with conventional livestock farming because of rapid growth rates and quick turnaround of animals to market. For broilers and pigs, the point at which BTFs were measured was selected pragmatically, at the post market-ready stage where the growth curve tends to level out. This is a practical rather than ideal point, but has the clear advantage of BTF determination at a stage where the animal tissues are consumed. For laying chickens, BTFs for eggs and chicken tissue were calculated approximately 2 months after the onset of laying, thus allowing a close approximation to a steady state. The input flux was an integral of all the significant daily dietary inputs which were primarily animal feeds, but included a component of the bedding materials that were inadvertently or intentionally ingested by the chickens, or soil that is known to be ingested during the course of rooting and foraging behaviour in the case of the outdoor pigs. Following previous studies (Fries et al., 1982; Hattermeyer-Frey and Travis, 1990; Fries, 1999; Foxall et al., 2004; Fernandes et al., 2011B), the bedding material intake was estimated at 2% of feed intake for chickens and the soil intake was estimated at

3.5% of feed intake for pigs. Water and veterinary supplements which were the other potential inputs were assessed as being less significant contributors to the input flux.

3.0 Results and Discussion

As would be expected from a study of this magnitude, the volume of raw data generated is very large, and has been presented in a sponsor report (Fera, 2017). This paper presents summaries of these results that are relevant to the understanding of contaminant occurrence and uptake from the recycled materials that were used in this study. As per convention, the concentrations of PCDD/Fs and dioxin-like PCBs have been summarised as toxic equivalents (WHO-TEQ), using the 2005 toxic equivalent factors (TEF_{2005} - van den Berg et al., 2006). The TEQ approach has also been extended to PBDD/Fs and PBBs using analogous TEF values to those used for PCDD/Fs, which is an interim measure until more suitable TEFs for these contaminants are developed. PCN concentrations are reported as the sum of the 12 measured PCN congeners. Although three of the HBCDD stereoisomers were measured, only α-HBCDD has been included in the summary as the β- and γ- forms were generally below detection limits (0.01 µg/kg) in the tissue samples.

3.1 Contaminant Occurrence in recycled materials

The concentrations of the contaminants in the recycled materials investigated in this study are summarised in Table 3.1. Apart from confirming the occurrence of the contaminants in the materials, this data also supports the diagnostic characterisation of the strength of evidence approach used in the study i.e. if a particular contaminant is detected in the tissue or eggs of the animal, but was not present in the material, then the detected occurrence cannot be attributed to the material. For the materials used in the chicken study, Table 3.1 shows that the recycled cardboard and DPS consistently had higher concentrations of contaminants. The low contamination levels in the clean wood shavings also confirms this material as an appropriate choice of control. The similarly low occurrence levels in recycled wood were surprising as some recycled wood materials are reported to show significant contamination (Rigby et al., 2015).

Contaminant concentrations in the recycled materials used in the pig module were more varied, with the biosolids generally showing higher contamination levels (apart from PCDD/Fs) than the ashes. This profile confirmed an expected characterisation, as the thermodynamic processes that lead to the production of the ashes would be expected to yield higher levels of PCDD/Fs. In fact the poultry litter ash concentrations (32.74 ng-WHO-TEQ/kg for the sum of PCBs and PCDD/Fs; with 97% contribution from PCDD/F TEQ) were in breach of the limit set under controls for PLA quality (Environment Agency, 2012). BDE-209 is reported separately to the sum EU10 PBDEs for two reasons. The commercial flame retardant “Deca” from which it derives was extensively used in the UK, and secondly, the restrictions on its use came into effect in 2008, much later than other PBDE commercial mixtures. It would thus be expected to persist to a later extent than the other PBDEs, which is evident from table 3.1.

3.2 Broiler chickens

Four groups of chickens were raised on four different bedding materials (Control wood shavings, recycled cardboard, DPS and recycled wood shavings) in individual segregated quadrants within the same housing. Contaminant occurrence in the tissues (muscle tissue, skin and liver) of these birds at the market ready stage are summarised in Table 3.2. Data for each of the analyte groups is presented in two ways – numerically, as paired data with the median concentration for the control samples always presented first followed by the median concentration for the recycled samples, and visually (e.g.$\uparrow$) as determined by the strength of evidence (see section 2.5). Chicken livers have low weight which limits the number of analytes that could be determined with sufficient sensitivity, so livers from each group were pooled to provide a composite sample. Visualisation is therefore not presented for livers, but highly divergent concentrations (differences >100% relative to control) are highlighted.

The recycled material that shows the most frequent evidence of effects is the DPS. There was strong evidence of uptake for ICES-6 PCBs, PBDEs and PCNs (median concentrations of 2.9 µg/kg, 5.1 µg/kg and 36 ng/kg respectively), in the muscle tissue. This observation was supported with some evidence of uptake in the skin, and elevated (highly divergent) concentrations in the liver (1.02/3.69 µg/kg, 2.1/6.1 µg/kg and 16/31 ng/kg control/recycled material, respectively for ICES-6 PCBs, PBDEs and PCNs). There is also some evidence of uptake in the tissue for PCDD/F and PCB TEQ, but this is not reflected in the skin or the liver. The recycled cardboard shows some evidence of uptake for the ICES-6 PCBs, both in the muscle tissue and the skin (concentrations of 0.73/0.91 µg/kg, 0.49/1.17 µg/kg, control/recycled material, respectively). Data for the recycled wood shavings showed no evidence of uptake for any of the analytes investigated. Thus, of the three recycled materials used, the strongest evidence of uptake lies with the DPS followed to some extent by the recycled cardboard. These observations are validated by the contaminant concentrations recorded for the recycled materials, with DPS showing highest concentrations among the three materials for PCBs, PBDEs and PCNs, followed by the recycled cardboard (Table 3.1).

3.3 Laying chickens

The four groups of laying chickens were raised using the same materials and the same housing as that used for the broilers. Chronologically, the study was carried out later, and relative to the shorter timescale to maturity for the broilers, the study period for the laying chickens from 1 day old chicks to study end was 7 months. In addition to the tissues, the eggs were also investigated, and data for this module is presented in Table 3.3, using the same format as that presented for the broilers. Similarly, no strength of evidence visualisation is given for the chicken livers because composite samples were analysed. This qualification also extends to the PFAS analysis in this module, as there was insufficient muscle and skin tissue left over for the analysis of individual birds, so these tissues were composited within groups to yield sufficient sample. Highly divergent concentrations (differences >100%) are highlighted for these tissues.

Effects were observed for all analytes, for all of the recycled materials. Strong evidence of uptake was observed in the eggs, for PCDD/F and PCB TEQ, ICES-6 PCBs, PBDEs, PFOS and PCNs (see Table 3.3). Although the strength of evidence analyses highlights all 3 materials, the magnitude of differences was strongest for the recycled cardboard and DPS. For the ICES-6 PCBs, the PBDEs and the PCNs, the median values for these materials exceeded control medians by more than an order of magnitude. Some evidence of uptake for these two materials was also observed for most of the other analytes except for α-HBCDD which was below the LOQ. These observations for the eggs are supported by some evidence of uptake in the muscle, skin and liver, in particular for the PCDD/F/PCB TEQ, ICES-6 PCBs, PBDEs and the PCNs. Among these tissues, liver shows the largest differences between control and recycled material. The recycled material concentrations in Table 3.1 are consistent with these tissue and egg concentration and validate the observations. It should be noted that although the paper-based materials did not show especially high concentrations of PFOS, the PFOS precursor chemicals, perfluoroalkyl phosphates which are commonly used in paper and packaging products, are reported to biotransform to PFOS (D'Eon and Maybury, 2007; Lee et al., 2010). The presence of these and other PFAS precursors may account for the PFOS levels observed in the tissues.

3.4 Outdoor pigs

Tissue concentration data for the outdoor pigs, measured at the conventionally accepted market ready state (animal weight of approx. 100 kg) of the animals is given in Table 3.4. Two types of tissue were analysed, muscle and liver, and although a natural variability in contaminant concentrations was observed for animals within the same treatment plot, the extent was greater for PCDD/Fs and PCBs in liver (standard deviation of 11 - 470% as compared to 7-24% for the muscle tissue). It is notable, although entirely expected, that the liver concentrations measured for all contaminants (except for HBCDD and TBBPA which were below the LOQ) were consistently higher than muscle tissue for all of the materials as well as the controls, and accords well with the physiology of contaminant distribution observed for liver in other studies (Olling et al., 1991; Fernandes et al., 2011B; Fernández-González et al., 2013). This has been observed for other species such as cattle and sheep as well and is not especially characteristic to this study. Data presentation and visualisation follows the same format as the poultry modules.

All of the measured contaminants were detected in both, muscle and liver tissue, except for HBCDD. The only contaminant where the strong evidence criteria was fulfilled for muscle tissue was PFOA in both of the ash materials, PLA and MBMA. However, this result could not be validated because PFOA was not detected in either of these two materials although the limit of detection for the measurement was a little high (<1 µg/kg). PFOA was detected in the soil, although the range of PFOA concentrations measured in the recycled material soils (table 3.1) for the duration of the study (0.3 - 0.9 µg/kg) were not significantly different to the PFOA concentrations in the control soils (0.6 - 0.8 µg/kg) and the concentration of PFOA in the treated soils would not have been significantly influenced by addition of ash containing < 1 µg/kg of PFOA. A similar outcome was evident for PFOS in muscle tissue which showed a weaker association with some evidence. Validated observations for muscle tissue were thus limited to some evidence for increased concentrations of ICES-6 PCBs and PBDEs. These were however, supported by results of strong evidence for PBDEs in PLA and the biosolids in the liver tissue. The biosolids, in particular, showed the highest incidence of strong evidence in this module for PBDEs, including BDE-209 and also for 'other PFAS' (sum of the measured PFAS compounds excluding PFOS and PFOA). These observations are validated by the high levels of occurrence of PBDEs and 'other PFAS' in the bio-solids (Table 3.1).

3.5 Discussion

In order to facilitate the recovery or recycling of waste materials for use as a resource, the end of waste process provides certain conditions that need to be satisfied for a specified material to cease to be classified as waste and be re-used (European Commission, 2018). These include lawful and specified purposes for the recycled product, commercial demand and most relevantly in the context of the current study, that it does not lead to adverse environmental or human health impacts. The principle purpose of this study is directly aligned with the last of these conditions, i.e. to investigate the potential of recycled bedding or fertilizer/soil-improver materials to provide a source of contaminant uptake into the tissues of farmed animals. Thus far. criteria for the material used in this study have not been laid down, but consideration is being given to waste paper and ash products.

The verification of contaminant occurrence in the materials (see section 3.1) prior to exposure to the animals was an essential part of this assessment, but occurrence in the other key input to the animals, namely feed, is equally important. Given the highly lipophilic properties of most of the contaminants under study, the intake of water (clean tap water was used) was not considered to be a significant source (Foxall et al., 2004; Fernandes et al., 2011B), and although veterinary care and advice was used during the study, no medicinal inputs were required. Thus the key sources to uptake could be attributed mainly to the feed and inadvertent ingestion of bedding in the case of the chickens, and feed together with the inadvertent intake of the material treated soil during foraging, by the pigs. The occurrence of key contaminants in the feed materials (starter, grower and where applicable, finisher) are shown in Table 3.1. These data demonstrate that contaminant concentrations in the feed were very low with most of the levels occurring below the limits of detection. Corresponding data for the soils used for the pig study, both before and after treatment with the recycled materials is also given in table 3.1. Occurrence levels prior to the study did not show any significant variation between the individual plots allocated to each of the materials, apart from a small elevation in PBDE levels in the control plot which were not apparent in the post spread samples. Concentrations were generally low apart from ‘other PFAS’ where combined concentrations were between 1.8 and 3.5 µg/kg. In general, contaminant concentrations in the soils either reduced marginally e.g. PCNs, or did not change substantially over the course of the study, apart from the PBDE concentrations for biosolid treated soil which showed an apparent increase over the course of the study. This could be caused by external environmental factors such as deposition but this is unlikely as only the single plot was affected. Additionally, the biosolid material showed relatively high levels of PBDEs. The increase may be related to the foraging behaviour of the pigs. As the recycled materials were initially ploughed into the soils they would occur to various depths within the upper soil surfaces. As the pigs grew older and stronger, it is likely that they were able to forage to greater depths, overturning and releasing more of the available material, thus resulting in higher concentration levels in the upper surfaces.

The weights of evidence approach used to assess whether any association existed between the inputs (including the recycled materials) and the resulting occurrence in animal tissues and eggs, indicates that some level of uptake was evident for all of the three studies, but to varying extents. The data in table 3.3 suggests that uptake was most evident, and for a larger number of contaminant classes (PCDD/Fs, PCBs, PBDEs, PCNs and PFOS), in the study on laying chickens. The broiler chickens were also exposed to the same materials, but fewer instances of strong evidence were observed for this module. Although other parameters e.g. genetic variation may play a part in this difference in response, there is another more practical and obvious variation in the conditions of the study. The timescale for the boiler chickens to grow from day-old to market readiness was 8 weeks, the corresponding timescale for the laying chickens from day-old to study-end was 7 months. Specifically, the time spent in contact with the recycled material, and hence the potential for ingestion/uptake and accumulation in tissues was far greater for the laying chickens and might be a more plausible explanation for the higher incidence of effects seen in this module. It is possible that this very reason (longer timescale) may play the opposite role in the case of the pigs. The timescale here from application of the recycled materials to the soil plots prior to sowing of the crop, to the market-ready state of the pigs was 13 months. Unlike the indoor, controlled environment of the chickens, the recycled materials remained in the exposed soil throughout this period, subjected to the removal (evaporation, bio- and photo-degradation, wind erosion, etc.) and dilution (within the upper soil layers) mechanisms that would affect the surface concentrations of the contaminants. Post spread soil concentrations are either similar or more generally, lower than pre-spread concentrations (apart from PBDEs as explained earlier). It is thus a possibility that the pigs were exposed to a lower level of contaminant exposure than might be expected from direct reference to the recycled material concentrations.

The conditions used for raising the pigs reflected those that were conventionally used by the farming industry, so the low incidence of effects seen in this module are likely to be realistic. However, one aspect of conventional farming that was not investigated in this study (out of scope due to cost and timescale limitations) was the repeated applications of materials to the same stretches of land used for raising pigs. The contaminants investigated here are environmentally persistent and some like the PCDD/Fs, PCBs, etc. have half-lives of several years (Milbrath et al., 2009). The potential build-up of contaminants in such re-used soils could result in very different exposure rates for the animals over the same time periods. Another un-investigated aspect of the study (also limited by cost restraints) was the batch-to-batch variation in contaminant concentrations of the recycled materials. Variation is highly likely because the production feed-stocks could arise from different sources (e.g. ash could be sourced from different incinerators at different times and feedstock conditions, biosolids could arise from different sewage treatment plants, etc.). The results of this study must therefore be seen in the light of these limitations.

It is important to note that the main focus of the study was on the potential uptake of contaminants by farm animals. However, given the attention in the study design to conventional farming practice in the raising of these animals and commercial availability of the recycled materials, it would be imprudent to ignore the food safety aspects of the results, and in particular, the resulting concentrations for the regulated contaminants, PCDD/F TEQ, PCDD/F + PCB TEQ and ICES-6 PCBs. The maximum tissue concentrations measured during the study along with the maximum concentrations specified by the existing regulations (European Commission, 2011) are presented in Table 3.5. These concentrations show that the resulting occurrence of the regulated contaminants in the vast majority of the tissues and eggs was well within the regulatory limits. A couple of samples of pig liver showed PCDD/F concentrations that were at the limit, but would not cross this threshold once measurement uncertainty was included. However, given the discussion in the previous paragraph, particularly the possibility of repeated application of material, further investigation would be prudent.

3.5.1 Bio-transfer

Although bio-transfer factors should be considered indicative rather than an absolute measure as mentioned earlier, they are a more versatile approach than either the bio-concentration factors (BCFs) or carry-over rates (CORs) (Fries et al., 1999), because they can relate contaminant concentration in animal tissues to the input flux of total dietary intake of PCDD/Fs, PCBs and other contaminants. Initially BTFs were estimated for all PCDD/F and PCB congeners, but as it would be impractical to include the full set, a smaller sub-set comprising the highest contributors to TEQ (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD 2,3,4,7,8-PeCDF, 123478-HxCDF, 123678-HxCDF and 234678-HxCDF,PCB 126, PCB 118, PCB169) and indicator PCBs, PCB-153 and PCB-138 were selected for presentation as they would be representative of dioxin-like congeners and other widely measured PCBs. This choice of congeners also reflects literature observations (Fries et al., 1999; Thomas et al., 1999; Fernandes et al., 2011B) that transfer rates can vary based on the degree of chlorination and molecular configuration of the molecules. Additionally, it was more meaningful to select compounds that occurred more abundantly and with a greater frequency of occurrence as the inclusion of some compounds that often occurred below the limit of detection (e.g. PCB 123, 1,2,3,4,7,8,9-HpCDF) could introduce greater uncertainty to the discussion. However, in order to express a collective transfer factor for all congeners, average BTF values for the full set of PCDD/Fs and indicator & DL-PCBs are also included (Table 3.6).

A number of observations relating to the magnitude of the BTFs for different contaminant groups and differences between the species are evident from Table 3.6. The most striking observations are the differences in the magnitude of the BTFs between chickens and pigs, in particular for muscle tissue, with BTFs for chickens being considerably higher. There is a degree of similarity of BTF values between chicken tissues and also between laying chickens and broilers for the PCDD/Fs, but BTF magnitude is much greater for the PCBs in laying chickens. This is perhaps indicative of the nature of PCB uptake in chickens and the pharmacokinetics associated with the higher chemical stability of PCBs relative to PCDD/Fs. The higher average BTF values for indicator PCBs (Table 3.6) may support this view. However, other factors should also be considered such as the longer exposure experienced by laying chickens. The BTF values for pig tissues are characterised by the obvious differences between muscle and liver, and contrast with the similarity within these tissues observed for chickens. Within pig liver, BTFs for PCDFs are also considerably higher than PCDDs or PCBs. The difference reflects the relative occurrence levels for PCDD/Fs and PCBs in liver and muscle of pig and other terrestrial animals (Olling et al., 1991; Fernandes et al., 2008; 2011B; Fernández-González et al., 2013) and is likely a result of liver function in processing lipids and the pharmacokinetics of PCDD/F and PCB absorption in this organ. These observation on the range of BTF values for both pigs and chickens is consistent with those reported in earlier studies (Foxall et al., 2004; Fernandes et al., 2011).

4.0 Conclusions

The results of this study show the occurrence of a wide range of environmental contaminants in commercially available recycled materials that are used in farming. Uptake of these contaminants to animal tissues and eggs was observed in all three of the modular studies (broiler and laying chickens and outdoor pigs) but the extent varied depending on the species and the recycled material. Among the contaminants investigated, PCBs, PBDEs, PCDD/Fs, PCNs and PFAS showed the highest potential to transfer, and the effects were most pronounced in laying chickens. Some contaminants e.g. PBDD/Fs, showed low concentrations in the recycled materials, making it difficult to statistically evaluate potential transfer. Bio-transfer factors estimated for PCDD/Fs and PCBs showed a greater magnitude for chicken muscle tissue relative to pigs with the highest BTF values observed for PCBs in laying chickens. From a food safety point of view, the foods produced under the conditions of the study (which reflected current husbandry practices) were generally within regulatory limits. However, the study did raise important issues for follow-up consideration. In particular, the potential build-up of contamination levels in soils arising from the repeated applications of contaminated materials to the same stretches of land used e.g. for raising pigs. Similarly, longer periods of exposure than those studied here could potentially result in higher levels of contaminant uptake to tissues and eggs of exposed animals. Recycled materials are typically produced in batches and batch-to-batch variation in contaminant concentrations is highly likely as production feed-stocks could arise from different sources. Given the variability that this implies and the potential of higher levels of contamination from repeated application of material and longer exposure periods, further investigation is advisable.

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Figure 1. Schematic of the plots used for pig rearing with detail for a single plot shown on the RHS

Figure 2. Strength of evidence visualisation: Examples of strong **↑**, or some ↑, evidence

The Potential of Recycled Materials used in Agriculture to Contaminate Food through Uptake by Livestock.

A. R. Fernandes, I. R. Lake, A. Dowding, M. Rose, N. R. Jones, R. Petch F. Smith, S Panton.

Tables 3.1 to 3.6

Table 3.1 Contaminant occurrence in recycled materials and other potential input material

Potential contaminant sources	PCDD/F & PCB TEQ ng/kg	ΣICES 6 PCBs µg/kg	PBDEs Sum EC-10 µg /kg	BDE-209 µg /kg	PBDD/F & PBB TEQ ng/kg	PFOS µg/kg	PFOA µg/kg	PF Other µg/kg	*PCNs (Σ 12) ng/kg	α-HBCD µg/kg	β-HBCD µg/kg	ɣ-HBCD µg/kg
Recycled materials - Poultry												
Clean Wood Shavings (Control)	0.24	0.18	0.41	0.33	0.09	0.6	3	13	5.9	0.03	0.01	<0.06
Recycled cardboard	8.33	13.34	223	218	1.41	1.9	9	15	161.5	11.7	3.9	27
Dried paper sludge (DPS)	8.69	27.59	420	417	1.53	3.9	5	54	426.9	8.89	4.87	41
Recycled Wood shavings	0.18	0.19	0.35	0.27	0.07	<1.0	4	9	6.6	0.03	0.01	<0.01
Other inputs												
Chicken feed Starter	0.12	0.07	0.1	0.07	0.03	nm	nm	nm	nm	nm	nm	nm
Chicken feed Grower	0.05	0.07	0.09	0.06	0.03	nm	nm	nm	nm	nm	nm	nm
Recycled materials - Pigs												
Meat and Bonemeal Ash (MBMA)	7.89	0.4	0.84	0.72	0.42	<1.0	<1.0	7	23.9	<0.02	<0.01	0.01
Poultry Litter Ash (PLA)	32.74	0.44	0.23	0.13	0.16	<1.0	<1.0	7	54.8	<0.01	<0.01	<0.01
Biosolid	2.57	3.66	2536	2504	13.58	23	1.4	57	145	0.02	0.01	<0.35
Other Inputs												
Pig Feed Starter	0.05	0.18	0.12	0.09	0.04	0.19	0.12	1.94	1.04	0.01	<0.01	0.01
Pig Feed Grower	0.05	0.15	0.16	0.13	0.04	0.22	0.22	1.08 i	1.0	<0.01	<0.01	<0.01
Pig Feed Finisher	0.05	0.06	0.14	0.1	0.04	0.04	0.14	2.72 i	1.22	<0.01	<0.01	0.01
Pig Straw	0.17	0.25	0.52	0.42	0.07	nm	nm	nm	3.97	0.06	0.02	0.03
Pig soil pre-spread												
Control	1.03	0.36	1.5	1.39	0.17	0.46	0.6	1.91	10.83	0.04	0.01	0.04
MBMA	1.14	0.36	0.44	0.34	0.15	0.48	0.74	2.03	7.58	0.02	0.01	0.04
PLA	1.07	0.36	0.41	0.31	0.13	0.6	0.89	3.5	10.29	0.03	0.01	0.04
Biosolid	1.09	0.33	0.4	0.3	0.19	0.3	0.34	1.68	8.71	0.01	0.01	0.01
Pig soil post-spread												
Control	1.13	0.35	0.51	0.41	0.11	0.56	0.82	1.89	7.68	0.01	0.01	0.01
MBMA	1.09	0.37	0.6	0.46	0.11	0.51	0.68	1.78	7.48	0.02	0.01	0.01
PLA	1.12	0.32	0.42	0.33	0.14	0.51	0.67	2.48	6.99	0.01	0.01	0.01
Biosolid	1.2	0.32	9.47	9.33	0.16	0.55	0.83	2.03	8.7	0.01	0.01	0.01

i – indicative value. * Σ 12 PCNs include PCNs 52/60,53, 66/67, 68, 69, 71, 72, 73, 74 & 75. nm – not measured

Table 3.2 Contaminant *concentrations in broiler chicken tissues showing strong **↑** or some ↑ evidence of uptake from recycled material.

Recycled material **Tissue**	Cardboard **Meat**	Dried Paper pulp	Wood shavings	Cardboard **Skin**	Dried Paper pulp	Wood shavings	Cardboard **Liver**	Dried Paper pulp	Wood shavings
PCB +PCDD/F TEQ (ng/kg fat)	- 0.52, 0.41	↑ 0.52, 0.88	- 0.52, 0.62	- 0.56, 0.28	- 0.56, 0.32	- 0.56, 0.33	0.49, 0.49	0.49, 0.71	0.49, 0.39
ΣICES6 (µg/kg fat)	↑ 0.73, 0.91	**↑** 0.73, 2.93	- 0.73, 0.84	↑ 0.49, 1.17	↑ 0.49, 2.42	- 0.49, 0.33	1.02, 1.13	1.02, 3.69	1.02, 0.75
PBDEs Sum EC-10 (µg/kg fat)	- 2.4, 1.63	**↑** 2.4, 5.1	- 2.4, 3.9	- 0.57, 0.85	↑ 0.57, 0.98	- 0.57, 0.67	2.1, 2.6	2.1, 6.1	2.1, 1.5
BDE-209 (µg/kg fat)	1.58, 1.42	1.58, 3.52	1.58, 2.69	0.33, 0.58	0.33, 0.75	0.33, 0.5	1.7, 2.2	1.7, 5.7	1.7, 1.2
PBDD/F & PBB TEQ (ng/kg fat)	- 4.1, 2.9	- 4.1, 2.2	- 4.1, 2.8	- 0.23, 0.28	- 0.23, 0.22	- 0.23, 0.42	0.73, 0.48	0.73, 0.57	0.73, 0.65
PFOS (µg/kg whole)	- 0.12, 0.07	- 0.12, 0.11	- 0.12, 0.06	nm	nm	nm	0.10, 0.07	0.10, 1.1	0.10, <0.05
PFOA (µg/kg whole)	- 0.14, 0.08	- 0.14, 0.04	- 0.14, 0.25	nm	nm	nm	0.13, 0.03	0.13, 0.08	0.13, 0.01
Sum, Other PFAS (µg/kg whole)	- 1.9, 1.2	- 1.9, 1.1	- 1.9, 1.0	nm	nm	nm	0.69, 0.31	0.69, 0.87	0.69, 0.27
PCNs (Sum 12) (ng/kg fat)	- 23, 19	**↑** 23, 36	- 23, 13	↑ 10, 18	↑ 10, 23	- 10, 6.0	16, 14	16, 31	16, 17
α-HBCD (µg/kg fat)	x	x	x	x	x	x	<0.42, <0.29	<0.42, <0.32	<0.42, <0.30

*- paired value comparison – first value is median for control, second is median value for recycled material. nm – not measured

Highly divergent concentrations

Table 3.3 Contaminant concentrations in layer chicken tissues/eggs showing strong **↑** or some ↑ evidence of uptake from recycled material.

Recycled material	Cardboard	Dried Paper pulp	Wood shavings	Cardboard	Dried Paper pulp	Wood shavings	Cardboard	Dried Paper pulp	Wood shavings	Cardboard	Dried Paper pulp	Wood shavings
Tissue	**Meat**			**Skin**			**Liver**			**Eggs**		
PCB +PCDD/F TEQ (ng/kg fat)	↑ 0.27, 0.85	↑ 0.27, 2.0	- 0.27, 0.5	↑ 0.20, 1.4	↑ 0.20, 1.7	- 0.20, 0.79	0.27, 1.5	0.27, 1.9	0.27, 0.46	**↑** 0.28, 0.88	**↑** 0.28, 1.6	**↑** 0.28, 0.46
ΣICES6 (µg/kg fat)	↑ 0.47, 5.95	↑ 0.47, 14.4	↑ 0.47, 1.21	↑ 0.39, 6.64	↑ 0.39, 11.0	- 0.39, 0.84	0.68, 10.8	0.68, 20.5	0.68, 1.09	**↑** 0.43, 5.04	**↑** 0.43, 14.9	**↑** 0.43, 0.98
PBDEs Sum EC-10 (µg/kg fat)	↑ 1.6, 3.5	↑ 1.6, 10	- 1.6, 2.6	↑ 0.44, 2.9	↑ 0.44, 7.8	↑ 0.44, 1.4	3.4, 31	3.4, 13	3.4, 3.6	**↑** 1.0, 11	**↑** 1.0, 16	**↑** 1.0, 1.2
BDE-209 (µg/kg fat)	↑ 1.28, 2.46	↑ 1.28, 8.15	- 1.28, <2.4	↑ 0.24, 1.63	↑ 0.24, 6.28	↑ 0.24, 0.86	3.2, 29.3	3.2,12.1	3.2, 3.11	**↑** 0.92, 11	**↑** 0.92, 15	**↑** 0.92, 1.1
PBDD/F & PBB TEQ (ng/kg fat)	- 0.73, 1.1	- 0.73, 0.82	- 0.73, 0.75	↑ 0.29, 0.79	↑ 0.29, 1.0	↑ 0.29, 0.82	0.62, 0.74	0.62, 0.59	0.62, 0.43	↑ 0.26, 0.36	↑ 0.26, 0.36	- 0.26, 0.24
PFOS (µg/kg whole)	0.03,0.06	0.03, 0.12	0.03, 0.04	- 0.27, 0.28	- 0.27, 0.33	- 0.27, 0.19	0.22, 0.96	0.22, 0.51	0.22, 0.29	**↑** 0.09, 0.60	**↑** 0.09, 1.5	- 0.09, 0.09
PFOA (µg/kg whole)	0.02,0.06	0.02, 0.05	0.02, 0.04	- 0.60, 0.69	- 0.60, 0.51	- 0.60, 0.48	0.07, 0.10	0.07, 0.09	0.07, 0.07	↑ 0.04, 0.04	↑ 0.04, 0.06	- 0.04, 0.03
Sum, Other PFAS (µg/kg whole)	0.54,0.43	0.54, 0.75	0.54, 0.62	- 4.9, 4.3	- 4.9, 3.8	- 4.9, 5.1	1.7, 1.2	1.7, 1.1	1.7, 1.2	↑ 0.51, 0.75	↑ 0.51, 1.2	↑ 0.51, 0.69
PCNs (Sum 12) (ng/kg fat)	↑ 14, 81	↑ 14, 154	↑ 14, 22	↑ 7.4, 68	↑ 7.4, 74	↑ 7.4, 16	55, 120	55, 118	55, 12	**↑** 4.3, 57	**↑** 4.3, 137	**↑** 4.3, 8.7
α-HBCD (µg/kg fat)	↑	↑	↑	↑	↑	↑	<0.24, 1.4	<0.24, 1.1	<0.24, 21	x	x	x

	<0.28, 0.71	<0.28, 1.15	<0.28, 9.26	<0.06, 1.01	<0.06, 0.87	<0.06, 15.1		

Highly divergent concentrations

Table 3.4 Contaminant concentrations in pig tissues showing strong **↑** or some ↑ evidence of uptake from recycled material.

Recycled material	Meat and Bonemeal Ash	Poultry Litter Ash	Biosolid	Meat and Bonemeal Ash	Poultry Litter Ash	Biosolid
Tissue	**Meat**			**Liver**		
PCB +PCDD/F TEQ (ng/kg fat)	↑ 0.21, 0.28	- 0.21, 0.19	- 0.21, 0.20	↑ 6.49, 6.14	- 6.49. 4.33	- 6.49, 3.69
ΣICES6 (µg/kg fat)	- 0.46, 0.50	↑ 0.46, 0.63	↑ 0.46, 0.58	**↑** 0.99, 1.48	↑ 0.99, 1.39	- 0.99, 1.08
PBDEs Sum EC-10 (µg/kg fat)	- 0.39, 0.83	↑ 0.39, 0.72	↑ 0.39, 0.73	↑ 0.96, 1.4	**↑** 0.96, 1.4	**↑** 0.96, 2.5
BDE-209 (µg/kg fat)	- 0.23, 0.61	↑ 0.23, 0.57	↑ 0.23, 0.42	- 0.56, 0.51	↑ 0.56, 0.67	**↑** 0.56, 1.84
PBDD/F & PBB TEQ (ng/kg fat)	- 0.21, 0.18	- 0.21, 0.17	- 0.21, 0.17	↑ 0.61, 0.74	- 0.61, 0.67	- 0.61, 0.64
PFOS (µg/kg whole)	↑ 0.03, 0.06	↑ 0.03, 0.42	↑ 0.03, 0.36	- 3.36, 2.56	- 3.36, 1.71	↑ 3.36, 4.93
PFOA (µg/kg whole)	↑ 0.14,0.64	↑ 0.14, 0.43	↑ 0.14, 0.29	↓ 0.69, 0.40	- 0.69, 0.82	**↑** 0.69, 1.22
Sum, Other PFAS (µg/kg whole)	- 1.53, 1.24	↑ 1.53, 1.75	- 1.53, 2.08	- 1.71, 1.69	- 1.71, 1.65	**↑** 1.71, 2.71
PCNs (Sum 12) (ng/kg fat)	- 4.8, 3.8	- 4.8, 4.4	- 4.8, 3.9	↑ 22, 26	↑ 22, 26	- 22, 16
α-HBCD (µg/kg fat)	x	x	x	x	x	x

Highly divergent concentrations

Table 3.5 Comparison of measured contaminant concentrations in produced food versus regulation limits

Food	Measured			Regulation limit (EC1881/2006 - amended 2016)		
	PCDD/F TEQ ng/kg fat	PCDD/F +PCB TEQ ng/kg fat	ICES-6 PCB µg/kg fat	PCDD/F TEQ ng/kg fat	PCDD/F +PCB TEQ ng/kg fat	ICES-6 PCB µg/kg fat
Broiler muscle	1.01	1.39	4.4	1.75	3	40
*Broiler liver (whole wt.)	0.03	0.05	0.13	0.3	0.5	3
Broiler skin	1.21	1.29	3.3	-	-	-
Eggs	0.85	1.92	18.2	2.5	5	40
Layer muscle	0.98	2.44	19.6	1.75	3	40
*Layer liver (whole wt.)	0.06	0.14	1.54	0.3	0.5	3
Layer skin	1.1	2.91	34.4	-	-	-
Pig muscle	0.36	0.41	0.9	1	1.25	40
*Pig liver(whole wt)	0.36	0.38	0.17	0.3	0.5	3

* Liver is regulated on a whole weight basis

Table 3.6 Estimated Bio-transformation factors (BTFs) for muscle tissue, skin, liver and eggs

	Broiler chickens			Laying chickens				Outdoor Pigs	
PCDD/Fs	Muscle	Skin	Liver	Muscle	Skin	Liver	Eggs	Muscle	Liver
2,3,7,8-TCDD	90	49	62	58	100	69	35	1.2	7.5
1,2,3,7,8-PeCDD	93	81	56	112	99	45	43	1.3	8.0
1,2,3,6,7,8-HxCDD	60	15	39	38	78	72	52	2.0	21
2,3,4,7,8-PeCDF	93	31	73	78	97	91	63	1.7	98
1,2,3,4,7,8-HxCDF	123	24	104	59	84	86	52	2.8	88
1,2,3,6,7,8-HxCDF	74	22	62	38	83	55	36	2.0	87
2,3,4,6,7,8-HxCDF	87	26	73	48	84	74	31	1.3	95
Average all PCDD/Fs	**110**	**33**	**92**	**47**	**79**	**61**	**38**	**1.4**	**46**
PCBs									
PCB118	41	40	37	146	169	206	171	1.8	4.9
PCB126	95	77	75	143	211	177	157	2.3	14
PCB169	136	49	50	84	186	97	78	3.6	6.3
PCB138	57	51	82	173	163	326	162	6.3	14
PCB153	107	60	59	220	216	271	194	6.3	14
Average Indicator and DL-PCBs	**49**	**31**	**42**	**111**	**125**	**135**	**105**	**2.1**	**6.2**
Average Indicator PCBs	**60**	**36**	**60**	**159**	**130**	**182**	**126**	**3.6**	**9.6**

The Potential of Recycled Materials used in Agriculture to Contaminate Food through Uptake by Livestock.

A. R. Fernandes, I. R. Lake, A. Dowding, M. Rose, N. R. Jones, R. Petch F. Smith, S Panton.

Highlights

First study on animal uptake of toxic organic contaminants from recycled materials

Laying chickens and eggs show strong evidence of contaminant uptake

Biotransformation factors can vary between species and body compartments

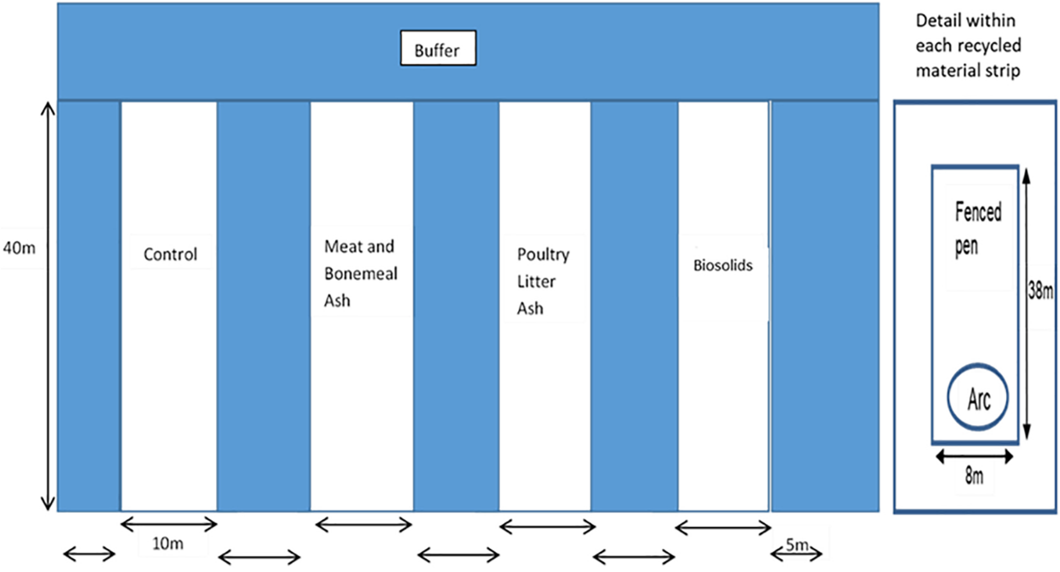
Buffer
Detail within each recycled material strip
40m
Control
Meat and Bonemeal Ash
Poultry Litter Ash
Biosolids
10m
5m
Fenced pen
Arc
38m
8m

Figure 1

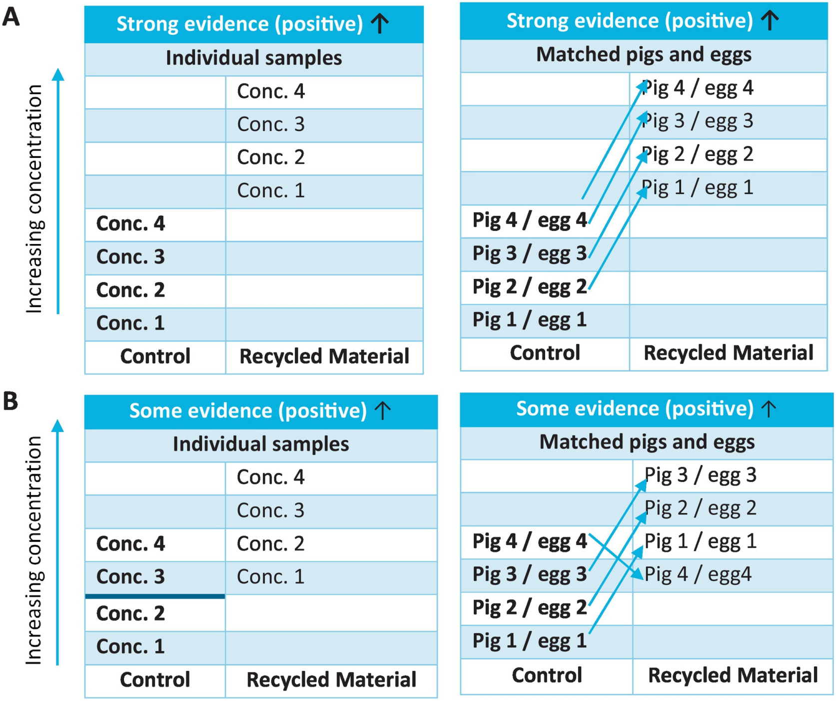
A
Strong evidence (positive) ↑
Individual samples
Increasing concentration
Conc. 4
Conc. 3
Conc. 2
Conc. 1
Conc. 4
Conc. 3
Conc. 2
Conc. 1
Control
Recycled Material
Strong evidence (positive) ↑
Matched pigs and eggs
Pig 4 / egg 4
Pig 3 / egg 3
Pig 2 / egg 2
Pig 1 / egg 1
Pig 4 / egg 4
Pig 3 / egg 3
Pig 2 / egg 2
Pig 1 / egg 1
Control
Recycled Material
B
Some evidence (positive) ↑
Individual samples
Increasing concentration
Conc. 4
Conc. 3
Conc. 4
Conc. 2
Conc. 3
Conc. 1
Conc. 2
Conc. 1
Control
Recycled Material
Some evidence (positive) ↑
Matched pigs and eggs
Pig 3 / egg 3
Pig 2 / egg 2
Pig 4 / egg 4
Pig 1 / egg 1
Pig 3 / egg 3
Pig 4 / egg4
Pig 2 / egg 2
Pig 1 / egg 1
Control
Recycled Material

Figure 2